Figure 1

Anti-CD3 WT

GATATCAAACTGCAGCAGTCAGGGGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCCT
GCAAGACTTCTGGCTACACCTTTACTAGGTACACGATGCACTGGGTAAAACAGAGGCCTGGACA
GGGTCTGGAATGGATTGGATACATTAATCCTAGCCGTGGTTATACTAATTACAATCAGAAGTTC
AAGGACAAGGCCACATTGACTACAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCC
TGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGATATTATGATGATCATTACTGCCTTGA
CTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCAGTCGAAGGTGGAAGTGGAGGTTCTGGT
GGAAGTGGAGGTTCAGGTGGAGTCGACGACATTCAGCTGACCCAGTCTCCAGCAATCATGTCTG
CATCTCCAGGGGAGAAGGTCACCATGACCTGCAGAGCCAGTTCAAGTGTAAGTTACATGAACTG
GTACCAGCAGAAGTCAGGCACCTCCCCCAAAAGATGGATTTATGACACATCCAAAGTGGCTTCT
GGAGTCCCTTATCGCTTCAGTGGCAGTGGGTCTGGGACCTCATACTCTCTCACAATCAGCAGCA
TGGAGGCTGAAGATGCTGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCACGTTCGG
TGCTGGGACCAAGCTGGAGCTGAAA

AA Sequence

DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKF
KDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSSVEGGSGGSG
GSGGSGGVDDIQLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQKSGTSPKRWIYDTSKVAS
GVPYRFSGSGSGTSYSLTISSMEAEDAATYYCQQWSSNPLTFGAGTKLELK

Fig. 2 A

VH2

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVRQAPGQGLEWIGYINPSR
GYTNYAQKLQGRVTMTTDTSTSTAYMELSSLRSEDTATYYCARYYDDHYCLDYWG
QGTTVTVSS

VH3

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVRQAPGQGLEWIGYINPSR
GYTNYAQKLQGRVTMTTDTSTSTAYLQMNSLKTEDTAVYYCARYYDDHYCLDYWG
QGTTVTVSS

VH5

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWIGYINPSR
GYTNYADSVKGRFTITTDKSTSTAYMELSSLRSEDTATYYCARYYDDHYCLDYWG
QGTTVTVSS

VH7

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWIGYINPSR
GYTNYNQKFKDRVTITTDKSTSTAYMELSSLRSEDTAVYYCARYYDDHYCLDYWG
QGTTVTVSS

Fig. 2 A (cont.)

VL1

DIQMTQSPSSLSASVGDRVTITCRASQSVSYMNWYQQKPGKAPKRWIYDT
SKVASGVPARFSGSGSGTDYSLTINSLEAEDAATYYCQQWSSNPLTFGGG
TKVEIK

VL2

DIVLTQSPATLSLSPGERATLSCRASQSVSYMNWYQQKPGKAPKRWIYDT
SKVASGVPARFSGSGSGTDYSLTINSLEAEDAATYYCQQWSSNPLTFGGG
TKVEIK

VL3

DIVLTQSPATLSLSPGERATLTCRASSSVSYMNWYQQKPGKAPKRWIYDT
SKVASGVPARFSGSGSGTDYSLTINSLEAEDAATYYCQQWSSNPLTFGGG
TKVEIK

Fig. 2 B

VH2

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACCTGGGGCCTCAGTGAAGGTGTCCTGC
AAGGCTTCTGGCTACACCGCTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCTGGACAGGGT
CTGGAATGGATTGGATACATTAATCCTAGCCGTGGTTATACTAATTACGCACAGAAGTTGCAGGGC
CGCGTCACAATGACTACAGACACTTCCACCAGCACAGCCTACATGGAACTGAGCAGCCTGCGTTCT
GAGGACACTGCAACCTATTACTGTGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGGGC
CAAGGCACCACGGTCACCGTCTCCTCA

VH3

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACCTGGGGCCTCAGTGAAGGTGTCCTGC
AAGGCTTCTGGCTACACCGCTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCTGGACAGGGT
CTGGAATGGATTGGATACATTAATCCTAGCCGTGGTTATACTAATTACGCACAGAAGTTGCAGGGC
CGCGTCACAATGACTACAGACACTTCCACCAGCACAGCCTACCTGCAAATGAACAGCCTGAAAACT
GAGGACACTGCAGTCTATTACTGTGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGGGC
CAAGGCACCACGGTCACCGTCTCCTCA

VH5

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACCTGGGGCCTCAGTGAAGGTGTCCTGC
AAGGCTTCTGGCTACACCTTTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCTGGACAGGGT
CTGGAATGGATTGGATACATTAATCCTAGCCGTGGTTATACTAATTACGCAGACAGCGTCAAGGGC
CGCTTCACAATCACTACAGACAAATCCACCAGCACAGCCTACATGGAACTGAGCAGCCTGCGTTCT
GAGGACACTGCAACCTATTACTGTGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGGGC
CAAGGCACCACGGTCACCGTCTCCTCA

Fig. 2 B (cont.)

VH7

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACCTGGGGCCTCAGTGAAGGTGTCCTGC
AAGGCTTCTGGCTACACCTTTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCTGGACAGGGT
CTGGAATGGATTGGATACATTAATCCTAGCCGTGGTTATACTAATTACAATCAGAAGTTCAAGGAC
CGCGTCACAATCACTACAGACAAATCCACCAGCACAGCCTACATGGAACTGAGCAGCCTGCGTTCT
GAGGACACTGCAGTCTATTACTGTGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGGGC
CAAGGCACCACGGTCACCGTCTCCTCA

Fig. 2 B (cont.)

VL1

GACATTCAGATGACCCAGTCTCCATCTAGCCTGTCTGCATCTGTCGGGGACCGTGTCACCATCACC
TGCAGAGCCAGTCAAAGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAAGGCACCCAAA
AGATGGATTTATGACACATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCT
GGGACCGACTACTCTCTCACAATCAACAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA
CAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

VL2

GACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCTGTCTCCAGGGGAGCGTGCCACCCTGAGC
TGCAGAGCCAGTCAAAGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAAGGCACCCAAA
AGATGGATTTATGACACATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCT
GGGACCGACTACTCTCTCACAATCAACAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA
CAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

VL3

GACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCTGTCTCCAGGGGAGCGTGCCACCCTGACC
TGCAGAGCCAGTTCAAGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAAGGCACCCAAA
AGATGGATTTATGACACATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCT
GGGACCGACTACTCTCTCACAATCAACAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA
CAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

Fig. 2 C

vH CDR1

Wt anti-CD3	GYTFTRYTMH
VH2,3	GYTATRYTMH
VH5,7	GYTFTRYTMH

vH CDR2

WT anti-CD3,	
VH7	YINPSRGYTNYNQKFKD
VH5	YINPSRGYTNYADSVKG
VH2, 3	YINPSRGYTNYAQKLQG

vH CDR3

WT anti-CD3,	
VH2, 3, 5, 7	YYDDHYCLDY

vK CDR1

WT anti-CD3,	
VL3	RASSSVSYMN
VL1, 2	RASQSVSYMN

vK CDR2

WT anti-CD3,	
VL1, 2, 3	DTSKVAS

vK CDR3

WT anti-CD3,	
VL1, 2, 3	QQWSSNPLT

Fig. 2 D

vH CDR1

WT anti-CD3	GGCTACACCTTTACTAGGTACACGATG CAC
VH2,3	GGCTACACCGCTACTAGGTACACGATG CAC
VH5,7	GGCTACACCTTTACTAGGTACACGATG CAC

vH CDR2

WT anti-CD3, VH7	TACATTAATCCTAGCCGTGGTTATACT AATTACAATCAGAAGTTCAAGGAC
VH5	TACATTAATCCTAGCCGTGGTTATACT AATTACGCAGACAGCGTCAAGGGC
VH2,3	TACATTAATCCTAGCCGTGGTTATACT AATTACGCACAGAAGTTGCAGGGC

VH CDR3

WT anti-CD3, VH2, 3, VH5, 7	TATTATGATGATCATTACTGCCTT GACTAC

Fig. 2 D (cont.)

vK CDR1

WT anti-CD3, VL3	AGAGCCAGTTCAAGTGTAAGTTACATG AAC
VL1, 2	AGAGCCAGTCAAAGTGTAAGTTACATG AAC

vK CDR2

WT anti-CD3, VL1-3	ACACATCCAAAGTGGCTTCT

VK CDR3

WT anti-CD3, VL1-3	CAACAGTGGAGTAGTAACCCGCTCACG

Figure 3

A) anti-CD3 (VH2/VL1)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC
TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA
CCGCTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT
GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG
TGGTTATACTAATTACGCACAGAAGTTGCAGGGCCGCGTCA
CAATGACTACAGACACTTCCACCAGCACAGCCTACATGGAA
CTGAGCAGCCTGCGTTCTGAGGACACTGCAACCTATTACTG
TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG
GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT
AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA
CGACATTCAGATGACCCAGTCTCCATCTAGCCTGTCTGCAT
CTGTCGGGGACCGTGTCACCATCACCTGCAGAGCCAGTCAA
AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA
GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT
CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC
GACTACTCTCTCACAATCAACAGCTTGGAGGCTGAAGATGC
TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA
CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

B) anti-CD3 (VH2/VL1)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVRQAP
GQGLEWIGYINPSRGYTNYAQKLQGRVTMTTDTSTSTAYME
LSSLRSEDTATYYCARYYDDHYCLDYWGQGTTVTVSSGEGT
STGSGGSGGSGGADDIQMTQSPSSLSASVGDRVTITCRASQ
SVSYMNWYQQKPGKAPKRWIYDTSKVASGVPARFSGSGSGT
DYSLTINSLEAEDAATYYCQQWSSNPLTFGGGTKVEIK

Figure 3

C) anti-CD3 (VH2/VL2)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAA-
AACCTGGGGCCTCAGTGAAGGTGTCCTG-
CAAGGCTTCTGGCTACACCGCTACTAGGTACACGATG-
CACTGGGTAAGGCAGGCACCTGGACAGGGTCTGGAATGGAT
TGGATACATTAATCCTAGCCGTGGTTATACTAATTACGCA-
CAGAAGTTGCAGGGCCGCGTCACAATGACTACAGA-
CACTTCCACCAGCACAGCCTACATGGAACTGAG-
CAGCCTGCGTTCTGAGGACACTGCAACCTATTACTGTGCAA
GATATTATGATGATCATTACTGCCTTGACTACTGGGGC-
CAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTAC-
TAGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCA-
GACGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCT
GTCTCCAGGGGAGCGTGCCACCCTGAGCTGCAGAGCCAGT-
CAAAGTGTAAGTTACATGAACTGGTACCAGCA-
GAAGCCGGGCAAGGCACCCAAAAGATGGATTTATGACA-
CATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGC
AGTGGGTCTGGGACCGACTACTCTCTCACAATCAA-
CAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA-
CAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGGGAC-
CAAGGTGGAGATCAAA

D) anti-CD3 (VH2/VL2)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVR-
QAPGQGLEWIGYINPSRGYTNY-
AQKLQGRVTMTTDTSTSTAYMELSSLRSEDTATYYCA-
RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADD
IVLTQSPATLSLSPGERATLSCRASQSVSYMNWYQQKPG-
KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-
DAATYYCQQWSSNPLTFGGGTKVEIK

Figure 3

E) anti-CD3 (VH2/VL3)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAA-
AACCTGGGGCCTCAGTGAAGGTGTCCTG-
CAAGGCTTCTGGCTACACCGCTACTAGGTACACGATG-
CACTGGGTAAGGCAGGCACCTGGACAGGGTCTGGAATGGAT
TGGATACATTAATCCTAGCCGTGGTTATACTAATTACGCA-
CAGAAGTTGCAGGGCCGCGTCACAATGACTACAGA-
CACTTCCACCAGCACAGCCTACATGGAACTGAG-
CAGCCTGCGTTCTGAGGACACTGCAACCTATTACTGTGCAA
GATATTATGATGATCATTACTGCCTTGACTACTGGGGC-
CAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTAC-
TAGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCA-
GACGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCT
GTCTCCAGGGGAGCGTGCCACCCTGACCTGCAGAGC-
CAGTTCAAGTGTAAGTTACATGAACTGGTACCAGCA-
GAAGCCGGGCAAGGCACCCAAAAGATGGATTTATGACA-
CATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGC
AGTGGGTCTGGGACCGACTACTCTCTCACAATCAA-
CAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA-
CAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGGGAC-
CAAGGTGGAGATCAAA

F) anti-CD3 (VH2/VL3)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVR-
QAPGQGLEWIGYINPSRGYTNY-
AQKLQGRVTMTTDTSTSTAYMELSSLRSEDTATYYCA-
RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADD
IVLTQSPATLSLSPGERATLTCRASSSVSYMNWYQQKPG-
KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-
DAATYYCQQWSSNPLTFGGGTKVEIK

Figure 4

A) anti-CD3 (VH3/VL1)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC
TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA
CCGCTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT
GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG
TGGTTATACTAATTACGCACAGAAGTTGCAGGGCCGCGTCA
CAATGACTACAGACACTTCCACCAGCACAGCCTACCTGCAA
ATGAACAGCCTGAAAACTGAGGACACTGCAGTCTATTACTG
TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG
GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT
AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA
CGACATTCAGATGACCCAGTCTCCATCTAGCCTGTCTGCAT
CTGTCGGGGACCGTGTCACCATCACCTGCAGAGCCAGTCAA
AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA
GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT
CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC
GACTACTCTCTCACAATCAACAGCTTGGAGGCTGAAGATGC
TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA
CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

B) anti-CD3 (VH3/VL1)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVR-
QAPGQGLEWIGYINPSRGYTNY-
AQKLQGRVTMTTDTSTSTAYLQMNSLKTEDTAVYYCARYYDD-
HYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADDIQMTQSP
SSLSASVGDRVTITCRASQSVSYMNWYQQKPG-
KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-
DAATYYCQQWSSNPLTFGGGTKVEIK

Figure 4

C) anti-CD3 (VH3/VL2)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC
TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA
CCGCTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT
GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG
TGGTTATACTAATTACGCACAGAAGTTGCAGGGCCGCGTCA
CAATGACTACAGACACTTCCACCAGCACAGCCTACCTGCAA
ATGAACAGCCTGAAAACTGAGGACACTGCAGTCTATTACTG
TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG
GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT
AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA
CGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCTGT
CTCCAGGGGAGCGTGCCACCCTGAGCTGCAGAGCCAGTCAA
AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA
GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT
CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC
GACTACTCTCTCACAATCAACAGCTTGGAGGCTGAAGATGC
TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA
CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

D) anti-CD3 (VH3/VL2)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVRQAP
GQGLEWIGYINPSRGYTNYAQKLQGRVTMTTDTSTSTAYLQ
MNSLKTEDTAVYYCARYYDDHYCLDYWGQGTTVTVSSGEGT
STGSGGSGGSGGADDIVLTQSPATLSLSPGERATLSCRASQ
SVSYMNWYQQKPGKAPKRWIYDTSKVASGVPARFSGSGSGT
DYSLTINSLEAEDAATYYCQQWSSNPLTFGGGTKVEIK

Figure 4

E) anti-CD3 (VH3/VL3)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAA-
AACCTGGGGCCTCAGTGAAGGTGTCCTG-
CAAGGCTTCTGGCTACACCGCTACTAGGTACACGATG-
CACTGGGTAAGGCAGGCACCTGGACAGGGTCTGGAATGGAT
TGGATACATTAATCCTAGCCGTGGTTATACTAATTACGCA-
CAGAAGTTGCAGGGCCGCGTCACAATGACTACAGA-
CACTTCCACCAGCACAGCCTACCTGCAAATGAACAGCCT-
GAAAACTGAGGACACTGCAGTCTATTACTGTGCAAGATATT
ATGATGATCATTACTGCCTTGACTACTGGGGCCAAGGCAC-
CACGGTCACCGTCTCCTCAGGCGAAGGTACTAG-
TACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGAC-
GACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCTGTC
TCCAGGGGAGCGTGCCACCCTGACCTGCAGAGCCAGTT-
CAAGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGG-
CAAGGCACCCAAAAGATGGATTTATGACACATCCA-
AAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGG
TCTGGGACCGACTACTCTCTCACAATCAACAGCTTG-
GAGGCTGAAGATGCTGCCACTTATTACTGCCAACAGTG-
GAGTAGTAACCCGCTCACGTTCGGTGGCGGGACCAAGGTG-
GAGATCAAA

F) anti-CD3 (VH3/VL3)

DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVR-
QAPGQGLEWIGYINPSRGYTNY-
AQKLQGRVTMTTDTSTSTAYLQMNSLKTEDTAVYYCA-
RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADD
IVLTQSPATLSLSPGERATLTCRASSSVSYMNWYQQKPG-
KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-
DAATYYCQQWSSNPLTFGGGTKVEIK

Figure 5

A) CD3 (VH5/VL1)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC
TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA
CCTTTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT
GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG
TGGTTATACTAATTACGCAGACAGCGTCAAGGGCCGCTTCA
CAATCACTACAGACAAATCCACCAGCACAGCCTACATGGAA
CTGAGCAGCCTGCGTTCTGAGGACACTGCAACCTATTACTG
TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG
GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT
AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA
CGACATTCAGATGACCCAGTCTCCATCTAGCCTGTCTGCAT
CTGTCGGGGACCGTGTCACCATCACCTGCAGAGCCAGTCAA
AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA
GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT
CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC
GACTACTCTCTCACAATCAACAGCTTGGAGGCTGAAGATGC
TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA
CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

B) CD3 (VH5/VL1)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAP
GQGLEWIGYINPSRGYTNYADSVKGRFTITTDKSTSTAYME
LSSLRSEDTATYYCARYYDDHYCLDYWGQGTTVTVSSGEGT
STGSGGSGGSGGADDIQMTQSPSSLSASVGDRVTITCRASQ
SVSYMNWYQQKPGKAPKRWIYDTSKVASGVPARFSGSGSGT
DYSLTINSLEAEDAATYYCQQWSSNPLTFGGGTKVEIK

Figure 5

C) anti-CD3 (VH5/VL2)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC
TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA
CCTTTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT
GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG
TGGTTATACTAATTACGCAGACAGCGTCAAGGGCCGCTTCA
CAATCACTACAGACAAATCCACCAGCACAGCCTACATGGAA
CTGAGCAGCCTGCGTTCTGAGGACACTGCAACCTATTACTG
TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG
GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT
AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA
CGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCTGT
CTCCAGGGGAGCGTGCCACCCTGAGCTGCAGAGCCAGTCAA
AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA
GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT
CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC
GACTACTCTCTCACAATCAACAGCTTGGAGGCTGAAGATGC
TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA
CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

D) anti-CD3 (VH5/VL2)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAP
GQGLEWIGYINPSRGYTNYADSVKGRFTITTDKSTSTAYME
LSSLRSEDTATYYCARYYDDHYCLDYWGQGTTVTVSSGEGT
STGSGGSGGSGGADDIVLTQSPATLSLSPGERATLSCRASQ
SVSYMNWYQQKPGKAPKRWIYDTSKVASGVPARFSGSGSGT
DYSLTINSLEAEDAATYYCQQWSSNPLTFGGGTKVEIK

Figure 5

E) anti-CD3 (VH5/VL3)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAA-
AACCTGGGGCCTCAGTGAAGGTGTCCTG-
CAAGGCTTCTGGCTACACCTTTACTAGGTACACGATG-
CACTGGGTAAGGCAGGCACCTGGACAGGGTCTGGAATGGAT
TGGATACATTAATCCTAGCCGTGGTTATACTAATTACG-
CAGACAGCGTCAAGGGCCGCTTCACAATCACTACAGACA-
AATCCACCAGCACAGCCTACATGGAACTGAG-
CAGCCTGCGTTCTGAGGACACTGCAACCTATTACTGTGCAA
GATATTATGATGATCATTACTGCCTTGACTACTGGGGC-
CAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTAC-
TAGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAG-
CAGACGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCT
CTGTCTCCAGGGGAGCGTGCCACCCTGACCTGCAGAGC-
CAGTTCAAGTGTAAGTTACATGAACTGGTACCAGCA-
GAAGCCGGGCAAGGCACCCAAAAGATGGATTTATGACA-
CATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGC
AGTGGGTCTGGGACCGACTACTCTCTCACAATCAA-
CAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGC-
CAACAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGG-
GACCAAGGTGGAGATCAAA

F) anti-CD3 (VH5/VL3)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVR-
QAPGQGLEWIGYINPSRGYTNY-
ADSVKGRFTITTDKSTSTAYMELSSLRSEDTATYYCA-
RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADD
IVLTQSPATLSLSPGERATLTCRASSSVSYMNWYQQKPG-
KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-
DAATYYCQQWSSNPLTFGGGTKVEIK

Figure 6

A) anti-CD3 (VH7/VL1)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC
TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA
CCTTTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT
GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG
TGGTTATACTAATTACAATCAGAAGTTCAAGGACCGCGTCA
CAATCACTACAGACAAATCCACCAGCACAGCCTACATGGAA
CTGAGCAGCCTGCGTTCTGAGGACACTGCAGTCTATTACTG
TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG
GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT
AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA
CGACATTCAGATGACCCAGTCTCCATCTAGCCTGTCTGCAT
CTGTCGGGGACCGTGTCACCATCACCTGCAGAGCCAGTCAA
AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA
GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT
CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC
GACTACTCTCTCACAATCAACAGCTTGGAGGCTGAAGATGC
TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA
CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

B) anti-CD3 (VH7/VL1)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVR-
QAPGQGLEWIGYINPSRGYT-
NYNQKFKDRVTITTDKSTSTAYMELSSLRSEDTAVYYCA-
RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADD
IQMTQSPSSLSASVGDRVTITCRASQSVSYMNWYQQKPG-
KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-
DAATYYCQQWSSNPLTFGGGTKVEIK

Figure 6

C) anti-CD3 (VH7/VL2)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAAAACC
TGGGGCCTCAGTGAAGGTGTCCTGCAAGGCTTCTGGCTACA
CCTTTACTAGGTACACGATGCACTGGGTAAGGCAGGCACCT
GGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGCCG
TGGTTATACTAATTACAATCAGAAGTTCAAGGACCGCGTCA
CAATCACTACAGACAAATCCACCAGCACAGCCTACATGGAA
CTGAGCAGCCTGCGTTCTGAGGACACTGCAGTCTATTACTG
TGCAAGATATTATGATGATCATTACTGCCTTGACTACTGGG
GCCAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTACT
AGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCAGA
CGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCTGT
CTCCAGGGGAGCGTGCCACCCTGAGCTGCAGAGCCAGTCAA
AGTGTAAGTTACATGAACTGGTACCAGCAGAAGCCGGGCAA
GGCACCCAAAAGATGGATTTATGACACATCCAAAGTGGCTT
CTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACC
GACTACTCTCTCACAATCAACAGCTTGGAGGCTGAAGATGC
TGCCACTTATTACTGCCAACAGTGGAGTAGTAACCCGCTCA
CGTTCGGTGGCGGGACCAAGGTGGAGATCAAA

D) anti-CD3 (VH7/VL2)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVR-
QAPGQGLEWIGYINPSRGYT-
NYNQKFKDRVTITTDKSTSTAYMELSSLRSEDTAVYYCA-
RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADD
IVLTQSPATLSLSPGERATLSCRASQSVSYMNWYQQKPG-
KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-
DAATYYCQQWSSNPLTFGGGTKVEIK

Figure 6

E) anti-CD3 (VH7/VL3)

GACGTCCAACTGGTGCAGTCAGGGGCTGAAGTGAAAA-
AACCTGGGGCCTCAGTGAAGGTGTCCTG-
CAAGGCTTCTGGCTACACCTTTACTAGGTACACGATG-
CACTGGGTAAGGCAGGCACCTGGACAGGGTCTGGAATGGAT
TGGATACATTAATCCTAGCCGTGGTTATACTAATTACAAT-
CAGAAGTTCAAGGACCGCGTCACAATCACTACAGACA-
AATCCACCAGCACAGCCTACATGGAACTGAG-
CAGCCTGCGTTCTGAGGACACTGCAGTCTATTACTGTGCAA
GATATTATGATGATCATTACTGCCTTGACTACTGGGGC-
CAAGGCACCACGGTCACCGTCTCCTCAGGCGAAGGTAC-
TAGTACTGGTTCTGGTGGAAGTGGAGGTTCAGGTGGAGCA-
GACGACATTGTACTGACCCAGTCTCCAGCAACTCTGTCTCT
GTCTCCAGGGGAGCGTGCCACCCTGACCTGCAGAGC-
CAGTTCAAGTGTAAGTTACATGAACTGGTACCAGCA-
GAAGCCGGGCAAGGCACCCAAAAGATGGATTTATGACA-
CATCCAAAGTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGC
AGTGGGTCTGGGACCGACTACTCTCTCACAATCAA-
CAGCTTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAA-
CAGTGGAGTAGTAACCCGCTCACGTTCGGTGGCGGGAC-
CAAGGTGGAGATCAAA

F) anti-CD3 (VH7/VL3)

DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVR-
QAPGQGLEWIGYINPSRGYT-
NYNQKFKDRVTITTDKSTSTAYMELSSLRSEDTAVYYCA-
RYYDDHYCLDYWGQGTTVTVSSGEGTSTGSGGSGGSGGADD
IVLTQSPATLSLSPGERATLTCRASSSVSYMNWYQQKPG-
KAPKRWIYDTSKVASGVPARFSGSGSGTDYSLTINSLEAE-
DAATYYCQQWSSNPLTFGGGTKVEIK

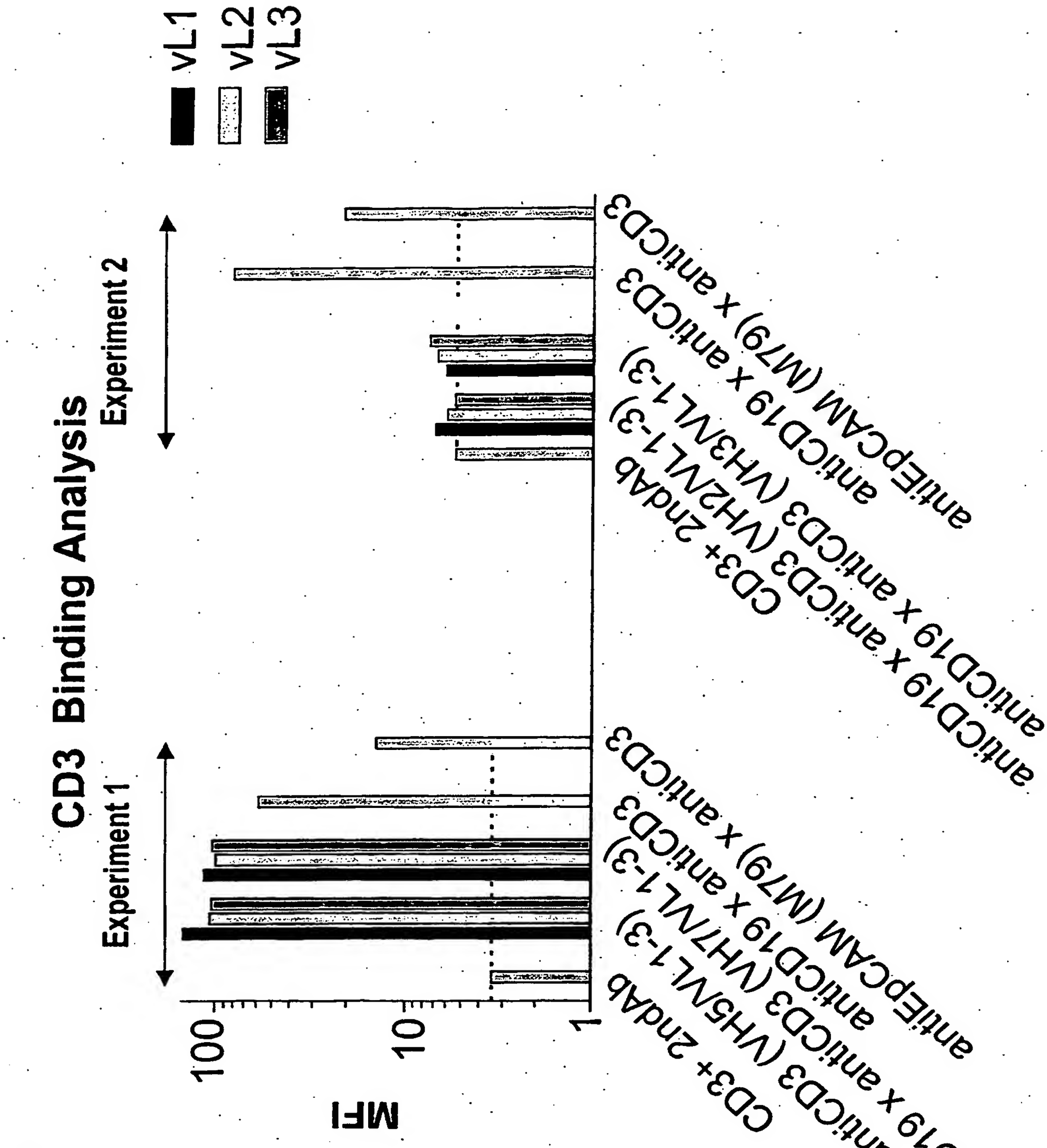

vL1
vL2
vL3
CD3 Binding Analysis
Experiment 2
Experiment 1
MFI
100
10
1
anti-EpCAM (M79) x anti-CD3
anti-CD19 x anti-CD3 (VH3VL 1-3)
anti-CD19 x anti-CD3 (VH2VL 1-3)
CD3+ 2ndAb
anti-CD19 x anti-CD3 (VH5VL 1-3)
anti-CD19 x anti-CD3 (VH7VL 1-3)
anti-EpCAM (M79) x anti-CD3
anti-CD19 x anti-CD3 (VH3VL 1-3)
anti-CD19 x anti-CD3 (VH2VL 1-3)
CD3+ 2ndAb

Figure 7A

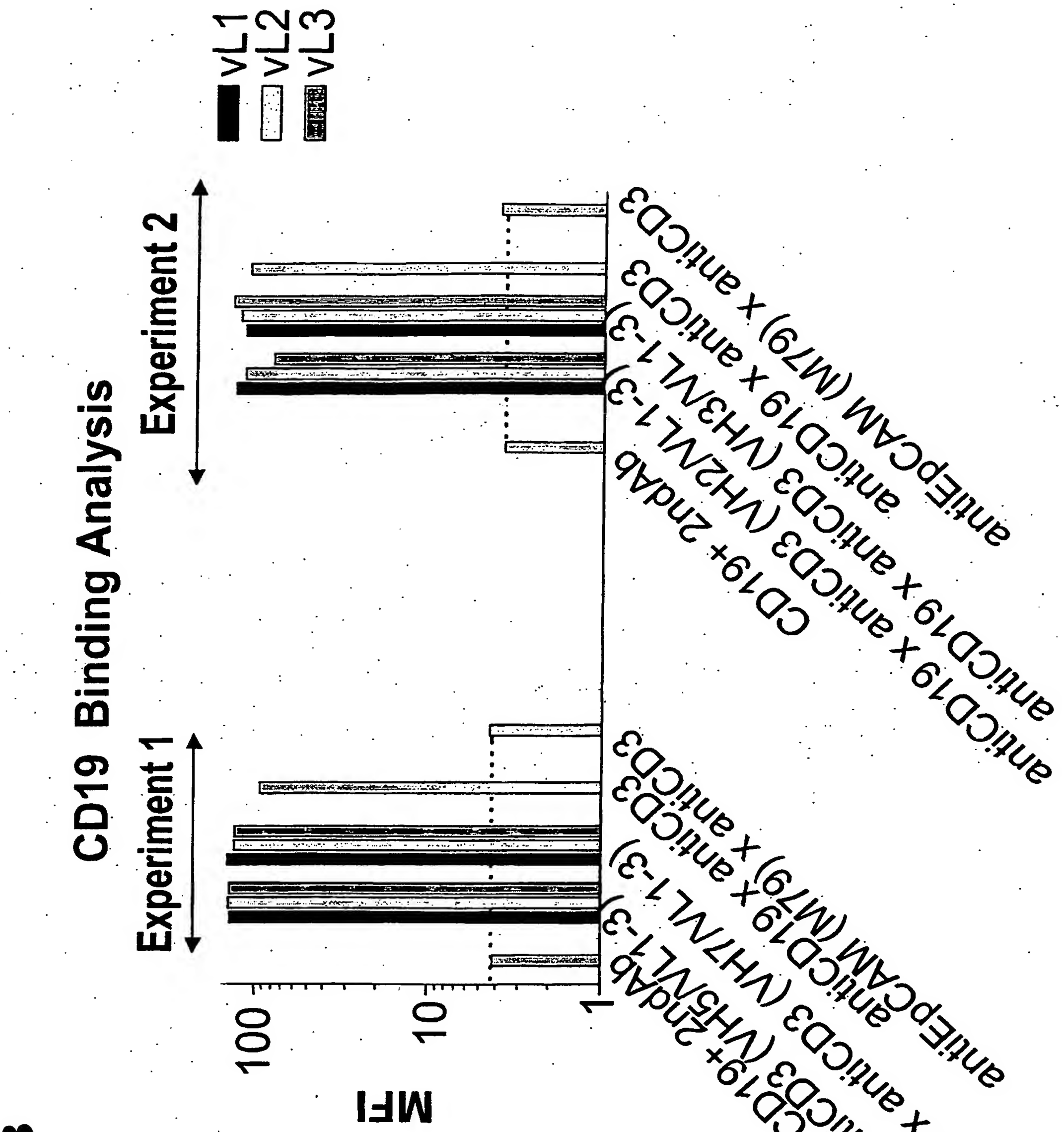

vL1
vL2
vL3
CD19 Binding Analysis
Experiment 2
Experiment 1
MFI
100
10
1
CD19+ 2ndAb
antiCD19 x antiCD3 (VH2VL1-3)
antiCD19 x antiCD3 (VH3VL1-3)
antiEpCAM (M79) x antiCD3
antiCD19 x antiCD3 (VH5VL1-3)
antiCD19 x antiCD3 (VH7VL1-3)

Figure 7B

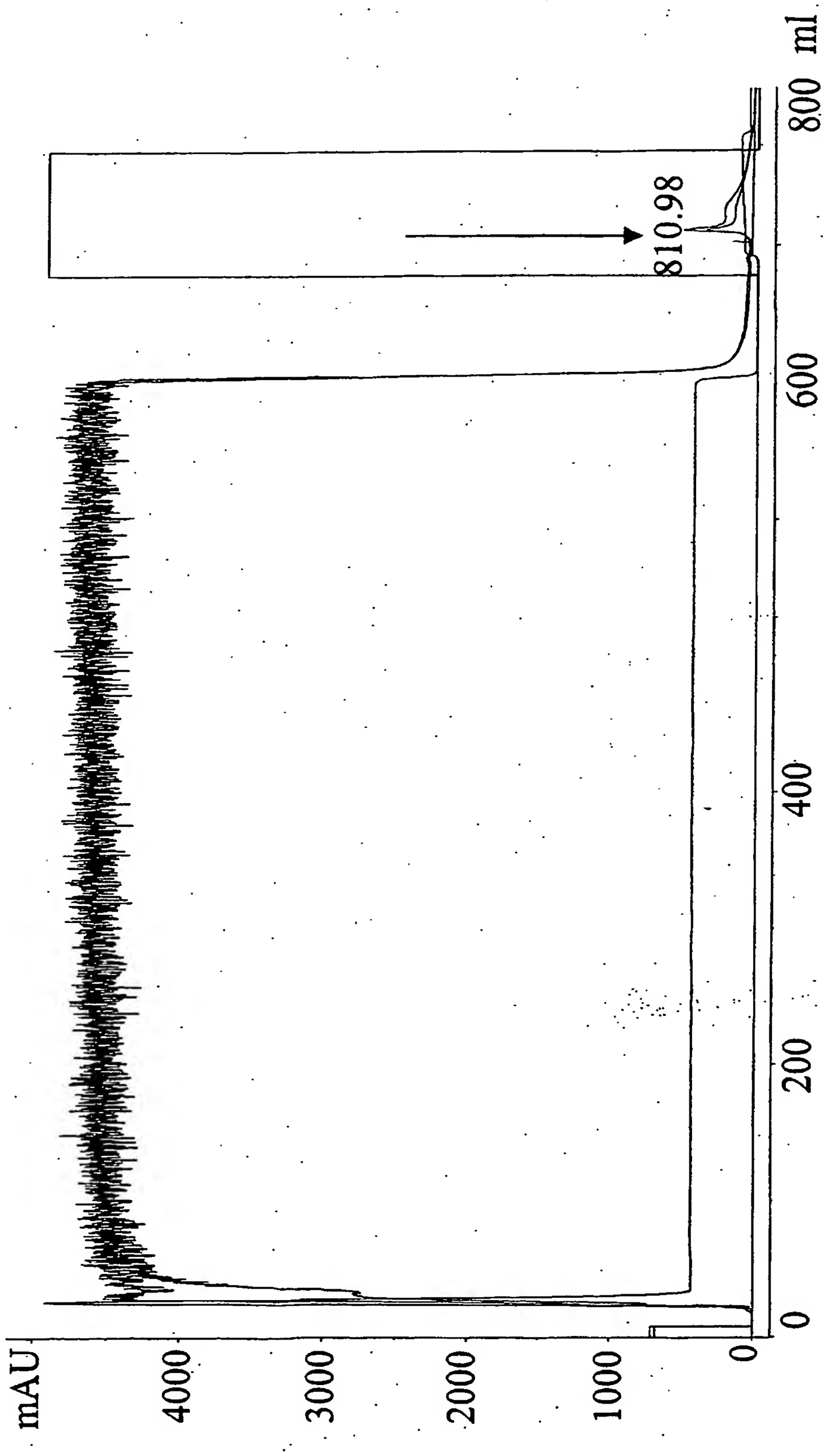


Figure 8

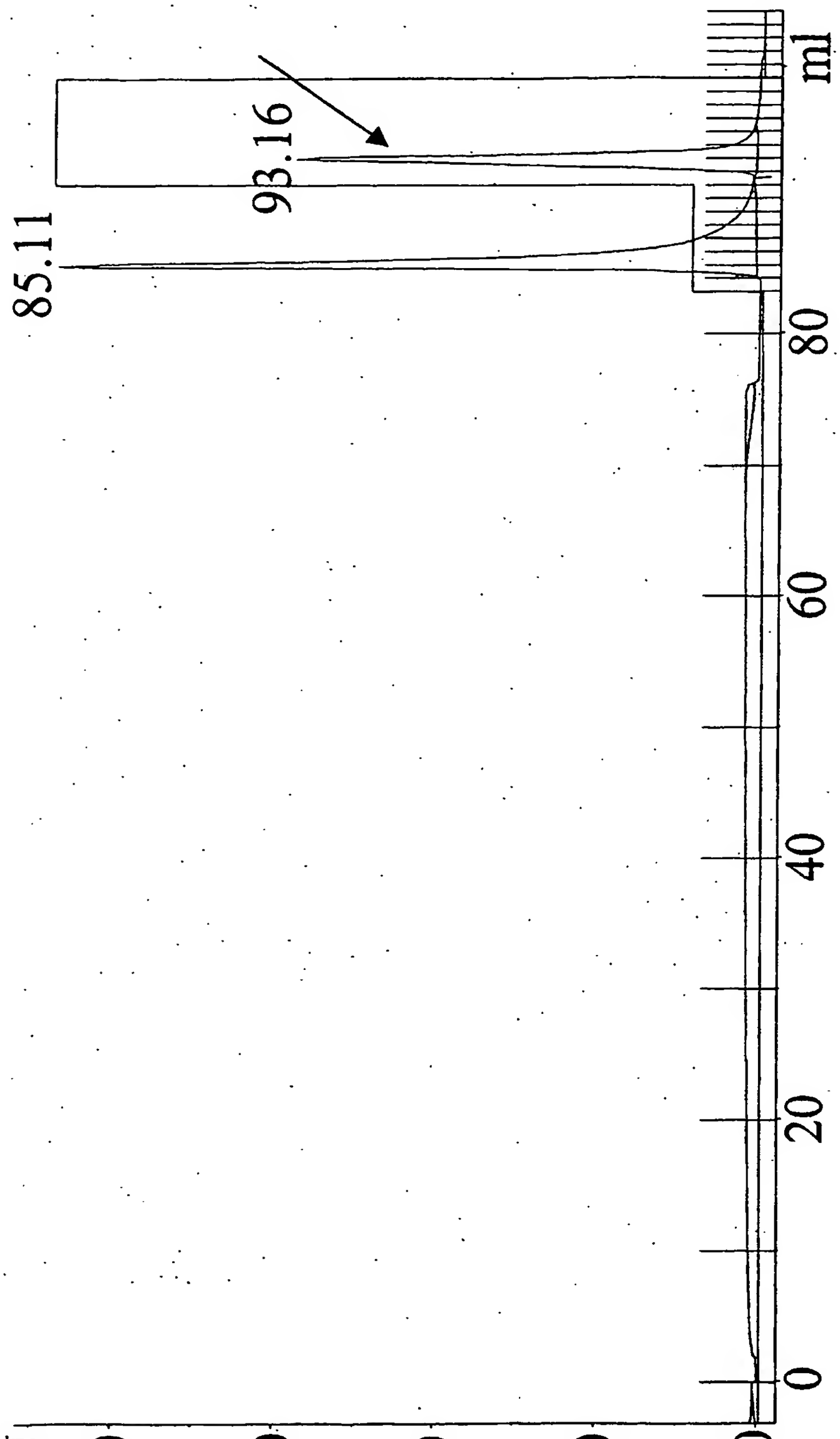


Figure 9

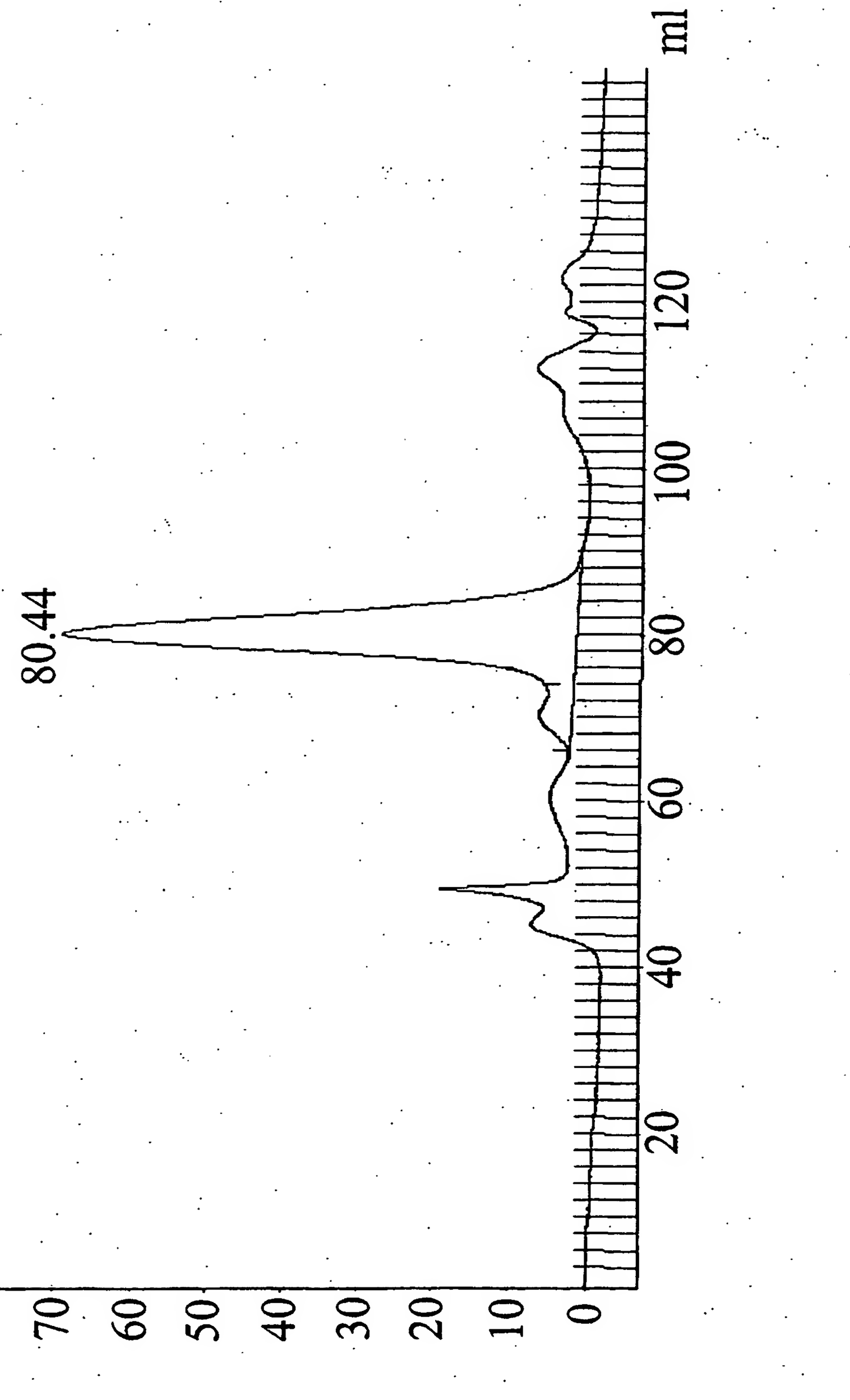
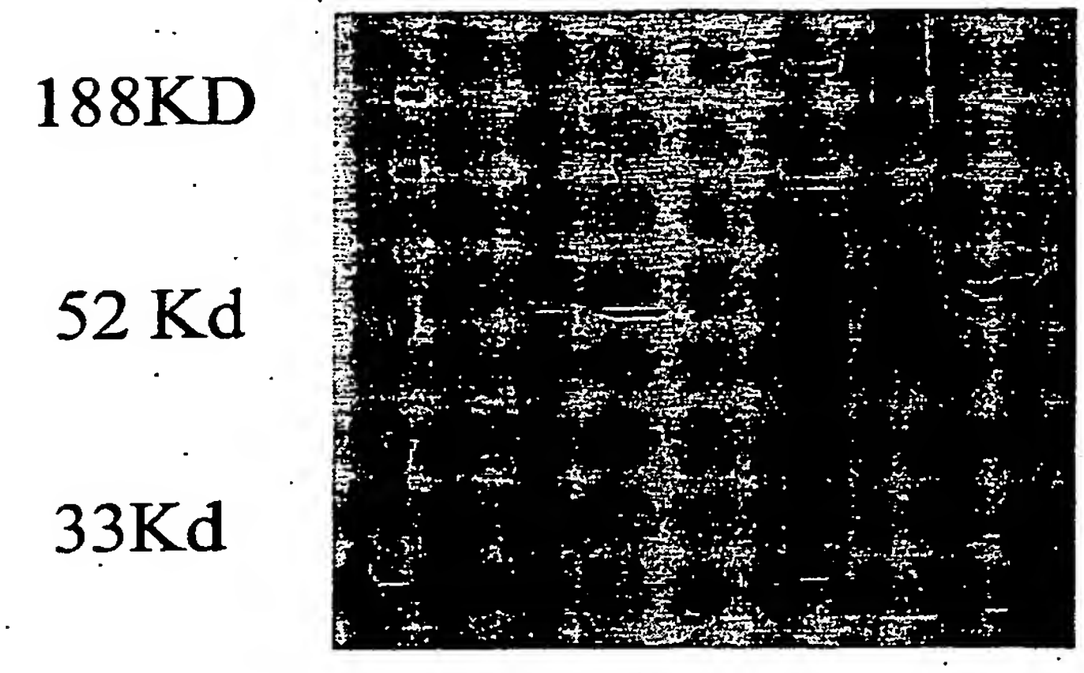


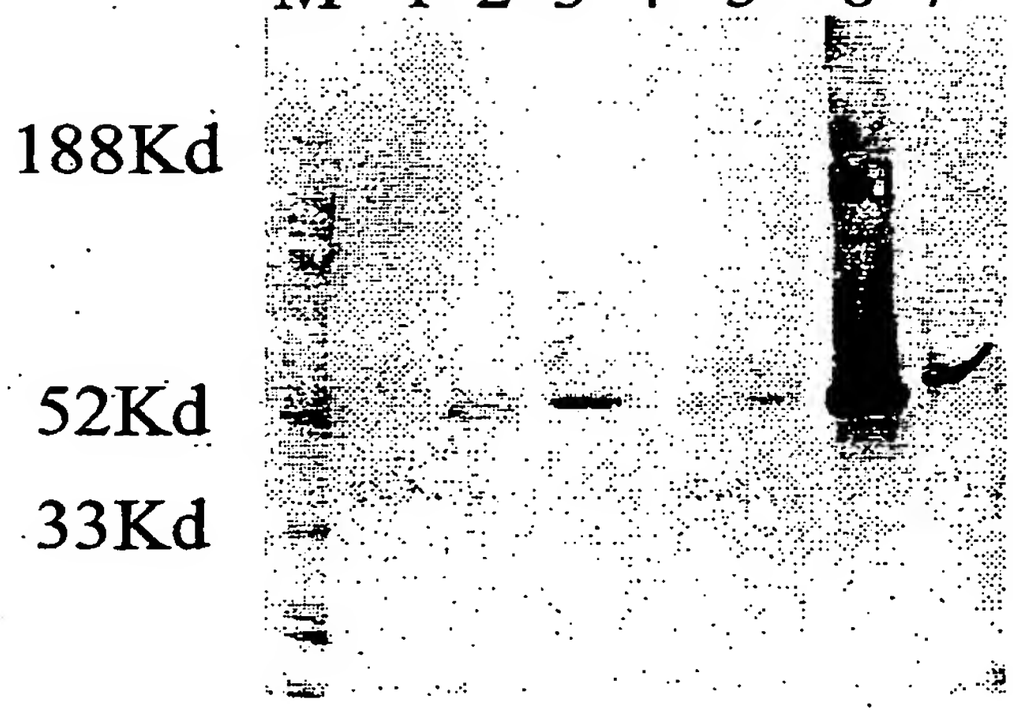
Figure 10

Figure 11

A)



B)



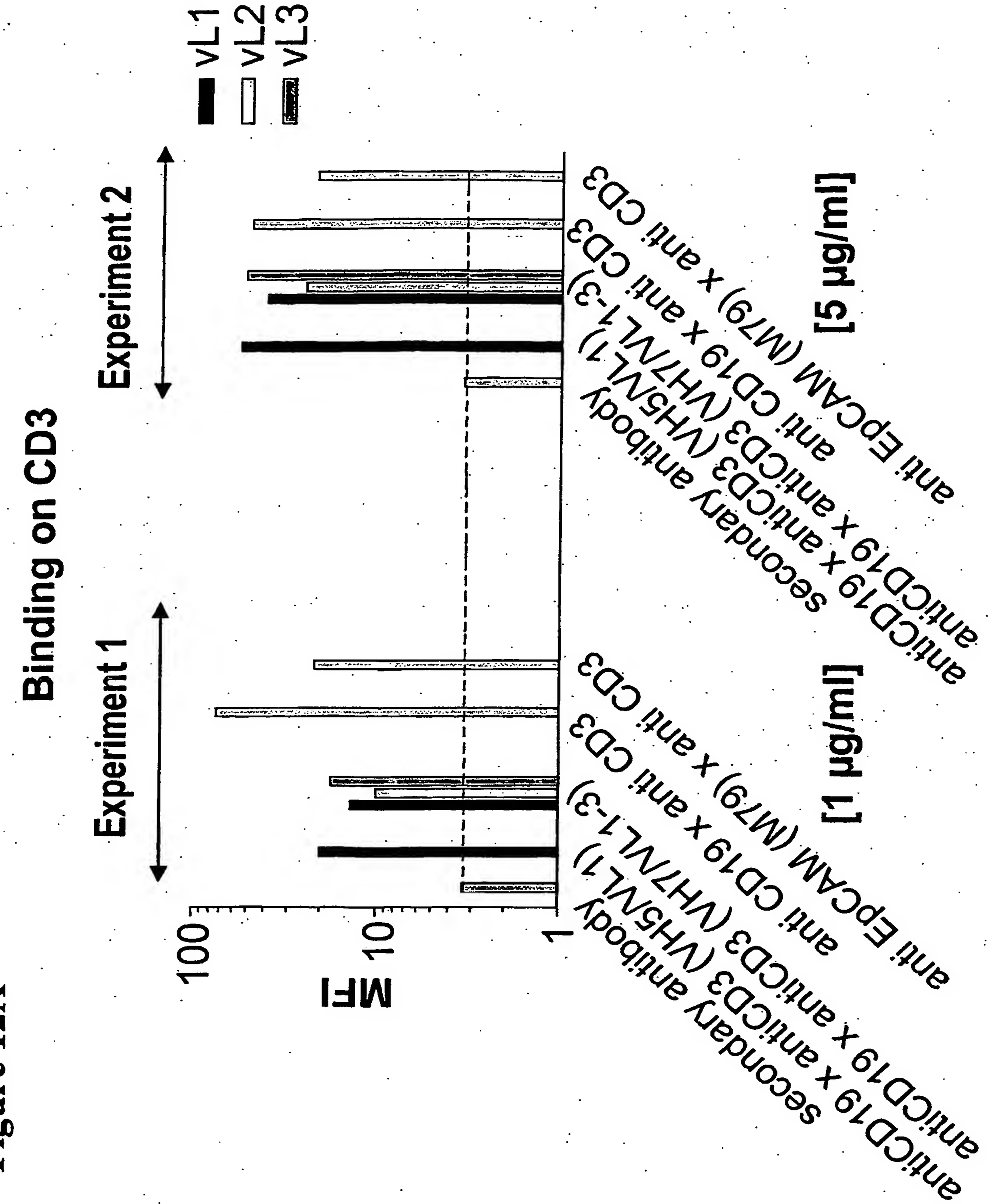

vL1
vL2
vL3
Experiment 2
Experiment 1
Binding on CD3
MFI
100
10
1
secondary antibody
antiCD19 x antiCD3 (VH5VL1)
antiCD19 x antiCD3 (VH7VL1-3)
anti EpCAM (M79) x anti CD3
[1 µg/ml]
[5 µg/ml]

Figure 12A

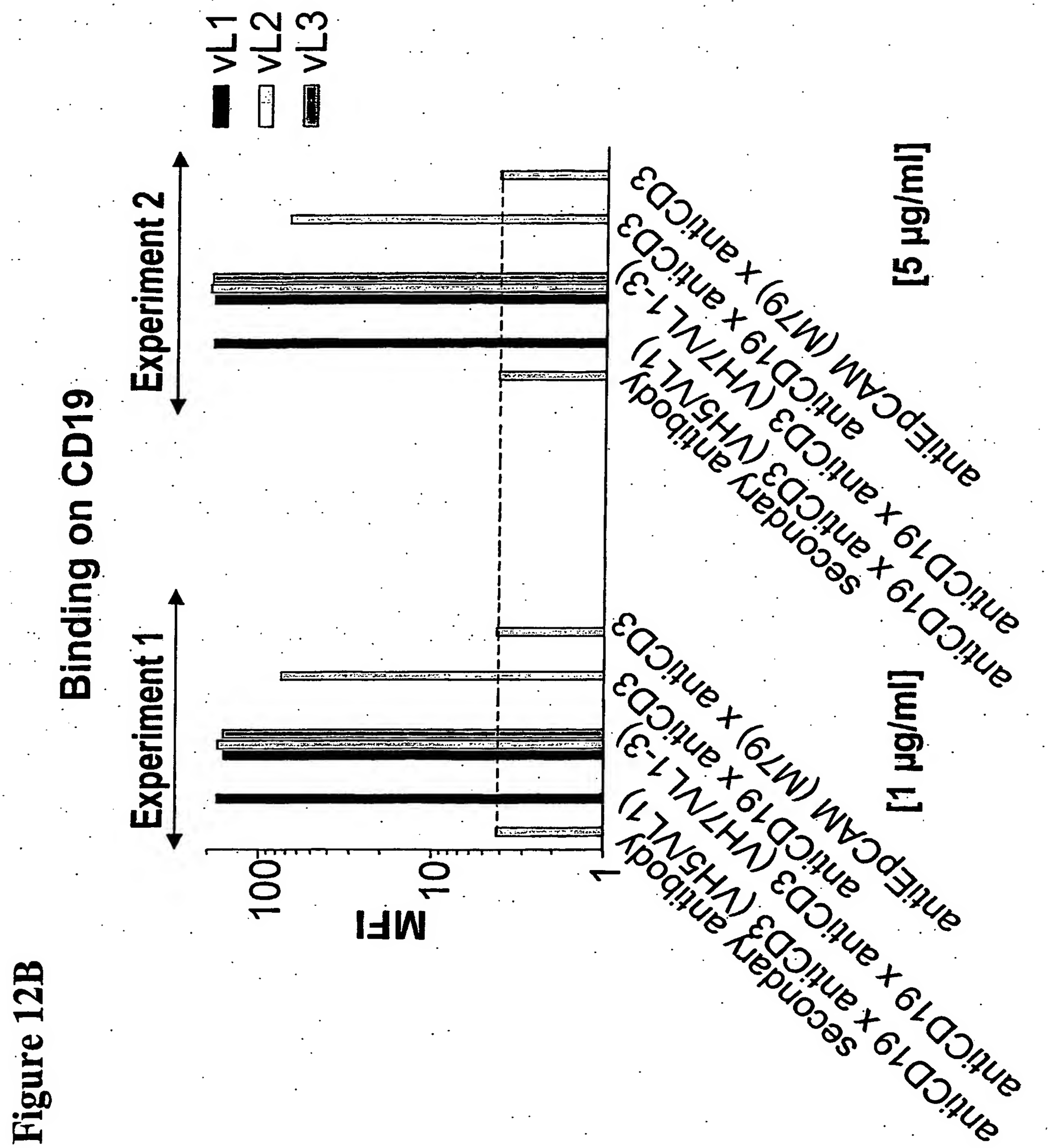

vL1
vL2
vL3
Binding on CD19
Experiment 2
Experiment 1
100
10
1
MFI
[1 µg/ml]
[5 µg/ml]
secondary antibody
antiCD19 x antiCD3 (VH5VL1)
antiCD19 x antiCD3 (VH7VL1)
antiEpCAM (M79) x antiCD3

Figure 12B

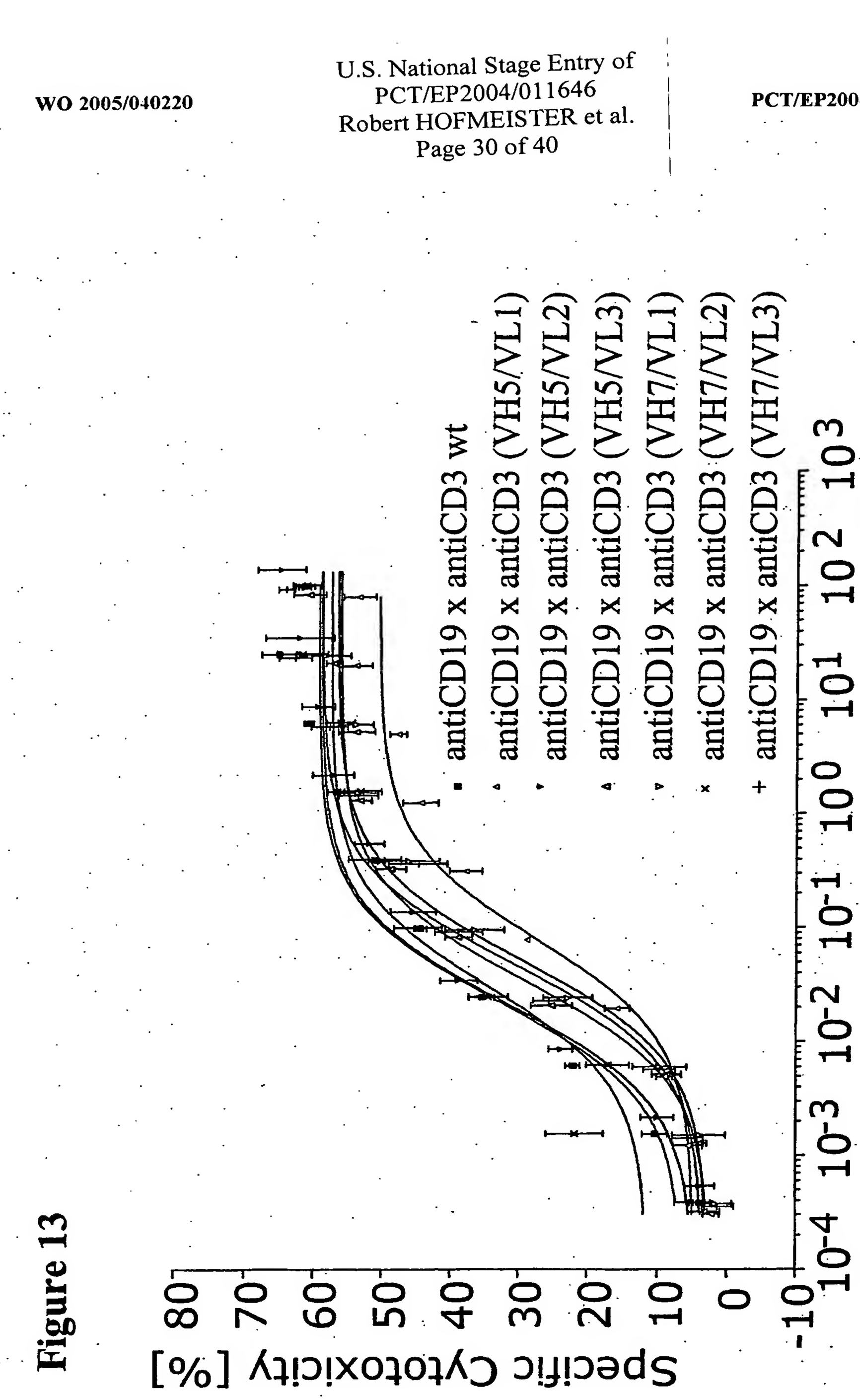
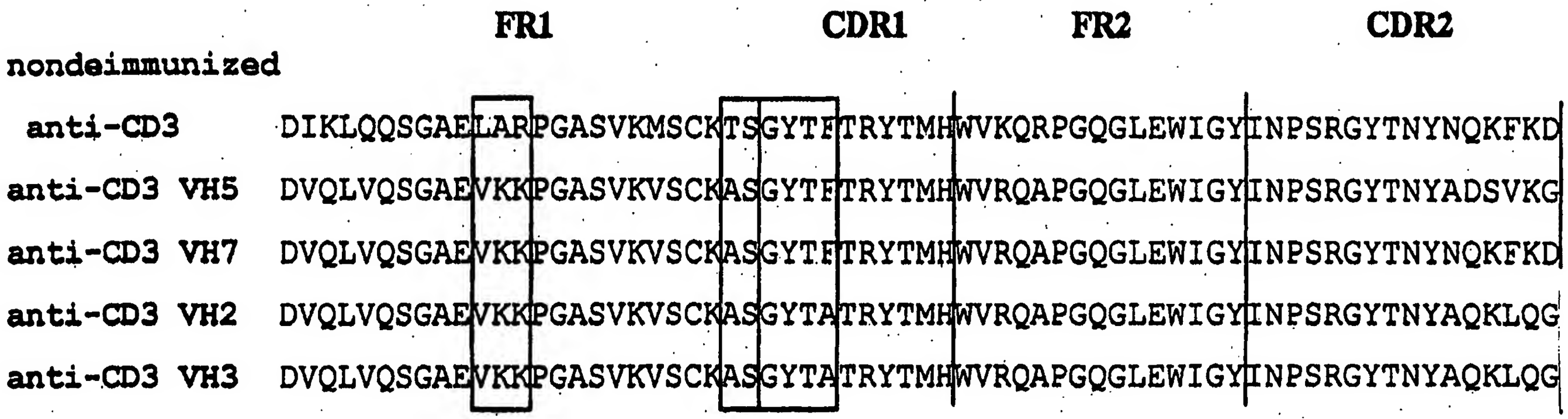

Specific Cytotoxicity [%]
80
70
60
50
40
30
20
10
0
-10
10-4
10-3
10-2
10-1
100
101
102
103
antiCD19 x antiCD3
Concentration [ng/ml]
antiCD19 x antiCD3 wt
antiCD19 x antiCD3 (VH5/VL1)
antiCD19 x antiCD3 (VH5/VL2)
antiCD19 x antiCD3 (VH5/VL3)
antiCD19 x antiCD3 (VH7/VL1)
antiCD19 x antiCD3 (VH7/VL2)
antiCD19 x antiCD3 (VH7/VL3)

Figure 13

Figure 14


FR1
CDR1
FR2
CDR2
nondeimmunized
anti-CD3 DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSRGYTNYNQKFKD
anti-CD3 VH5 DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWIGYINPSRGYTNYADSVKG
anti-CD3 VH7 DVQLVQSGAEVKKPGASVKVSCKASGYTFTRYTMHWVRQAPGQGLEWIGYINPSRGYTNYNQKFKD
anti-CD3 VH2 DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVRQAPGQGLEWIGYINPSRGYTNYAQKLQG
anti-CD3 VH3 DVQLVQSGAEVKKPGASVKVSCKASGYTATRYTMHWVRQAPGQGLEWIGYINPSRGYTNYAQKLQG

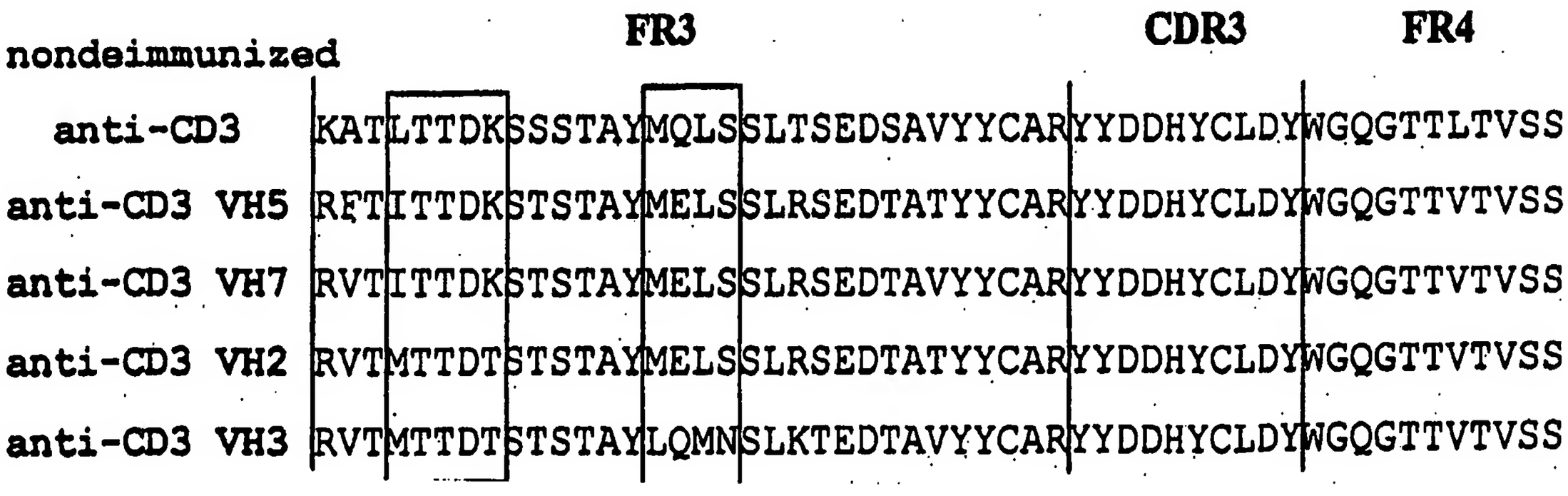
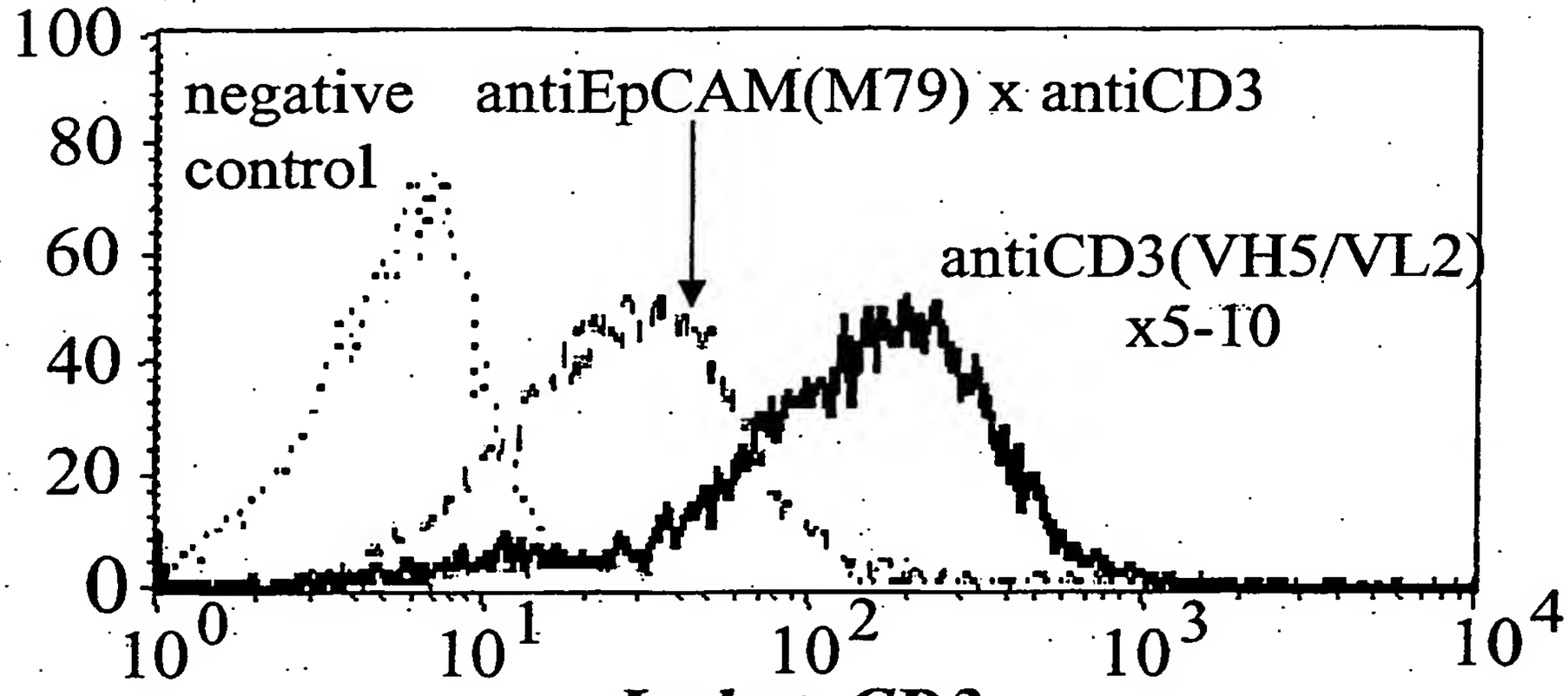
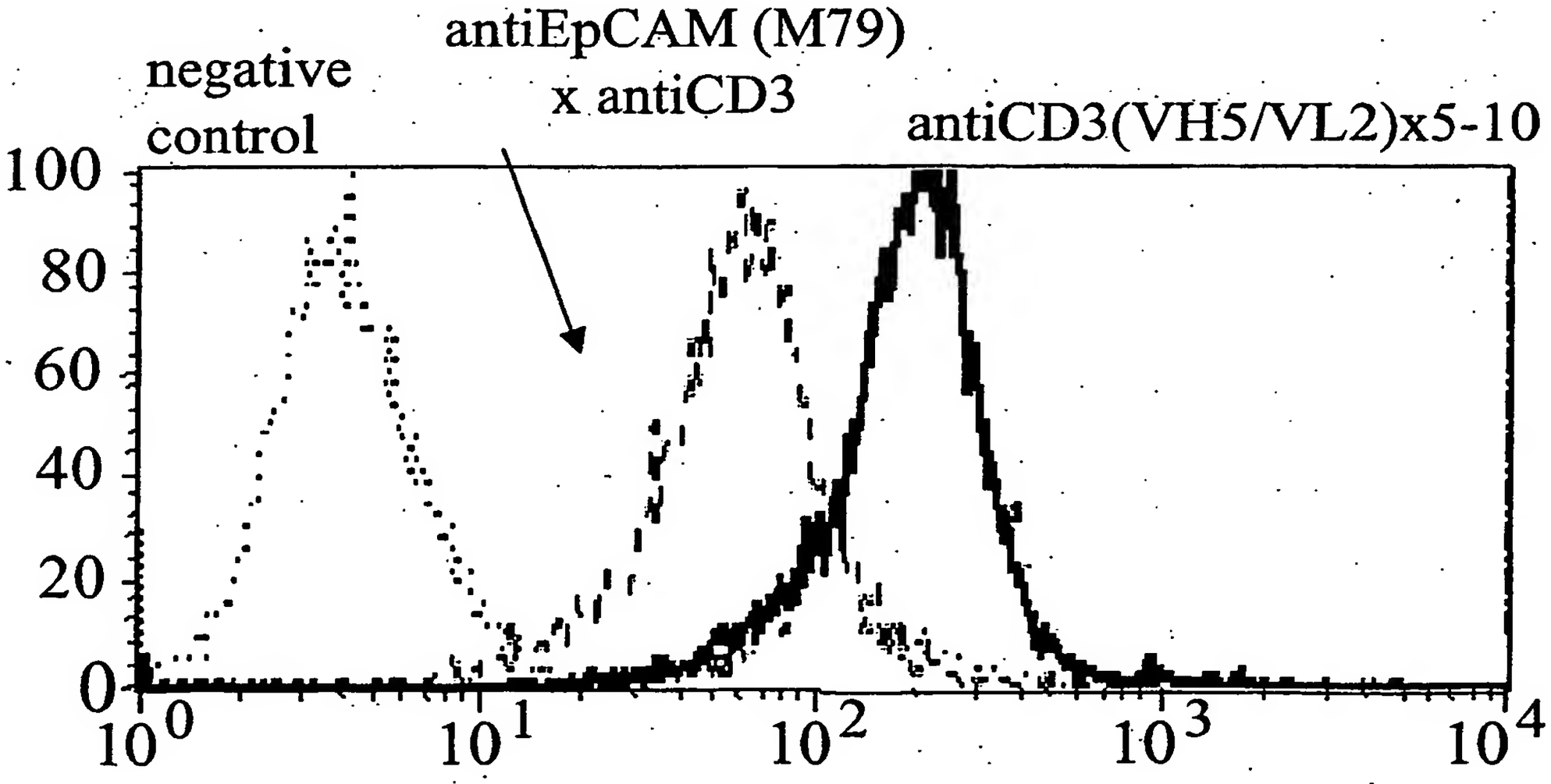

FR3
CDR3
FR4
nondeimmunized
anti-CD3 KATLTTDKSSSTAYMQLSSLTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSS
anti-CD3 VH5 RFTITTDKSTSTAYMELSSLRSEDTATYYCARYYDDHYCLDYWGQGTTVTVSS
anti-CD3 VH7 RVTITTDKSTSTAYMELSSLRSEDTAVYYCARYYDDHYCLDYWGQGTTVTVSS
anti-CD3 VH2 RVTMTTDTSTSTAYMELSSLRSEDTATYYCARYYDDHYCLDYWGQGTTVTVSS
anti-CD3 VH3 RVTMTTDTSTSTAYLQMNSLKTEDTAVYYCARYYDDHYCLDYWGQGTTVTVSS

Figure 15 A

antiCD3(VH5/VL2) x 5-10 (SEQ ID NO: 37)



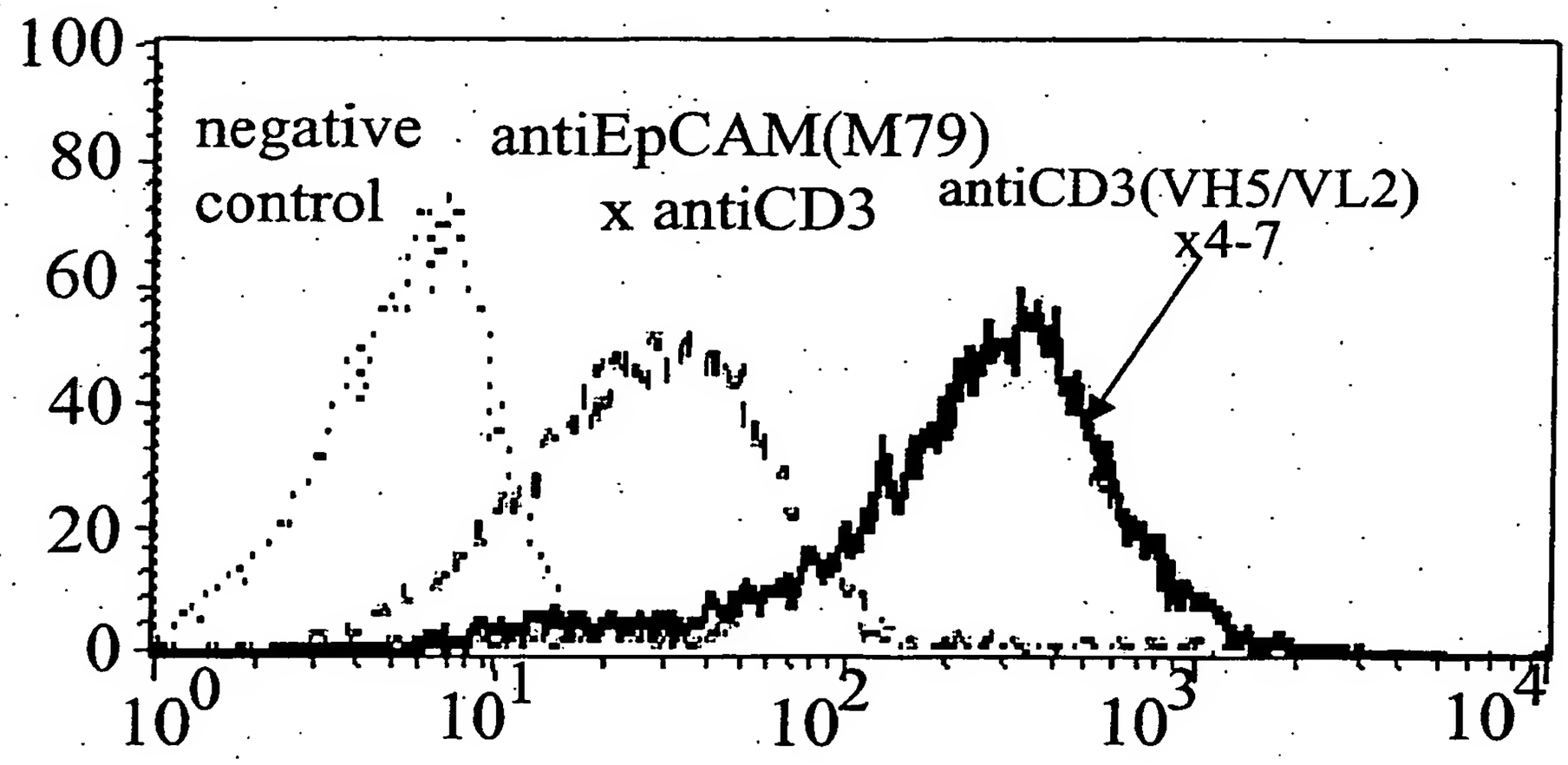
Jurkat, CD3



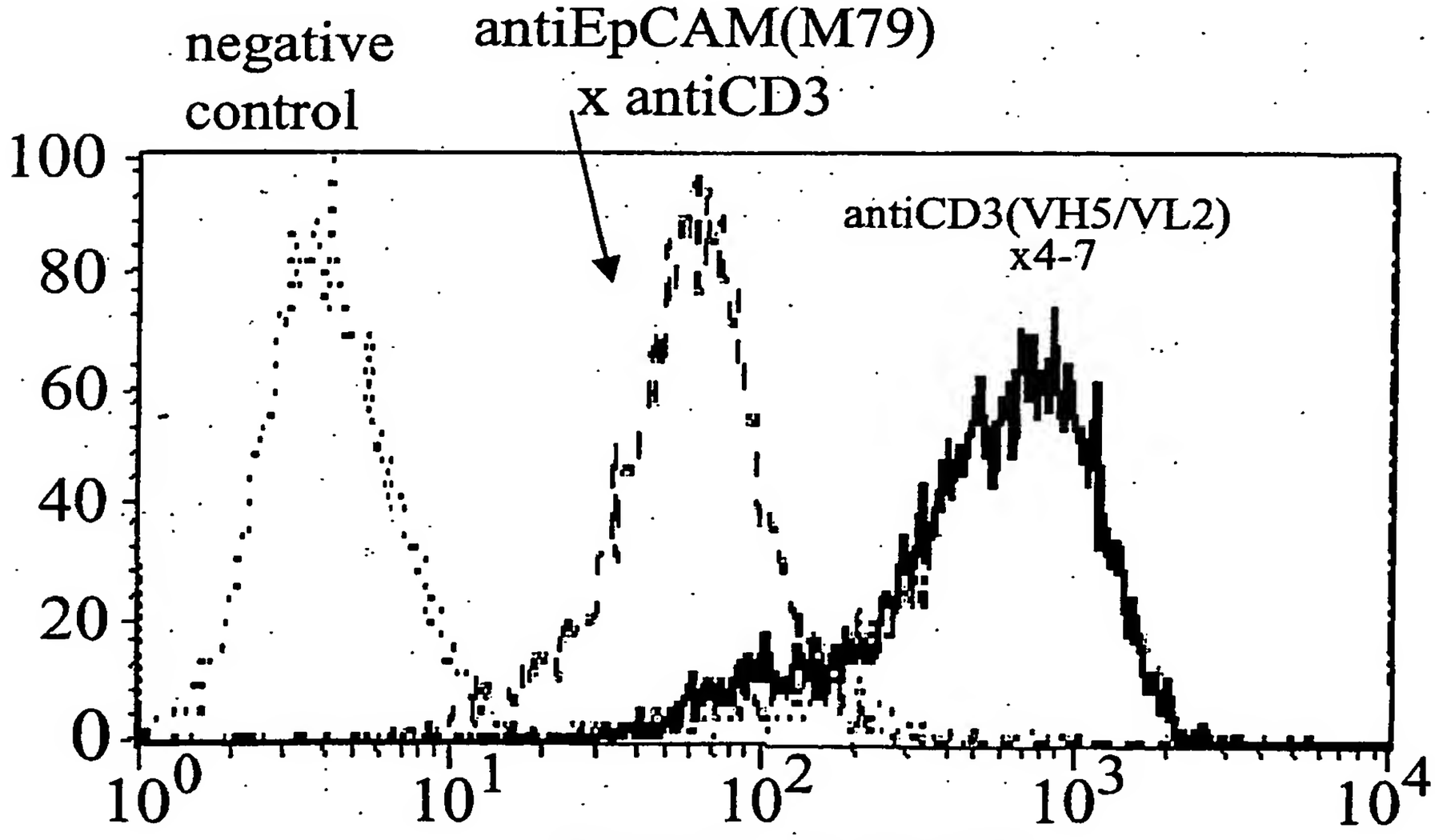
KatoIII, EpCAM

Figure 15B

antiCD3(VH5/VL2) x 4-7 (SEQ ID NO:33)



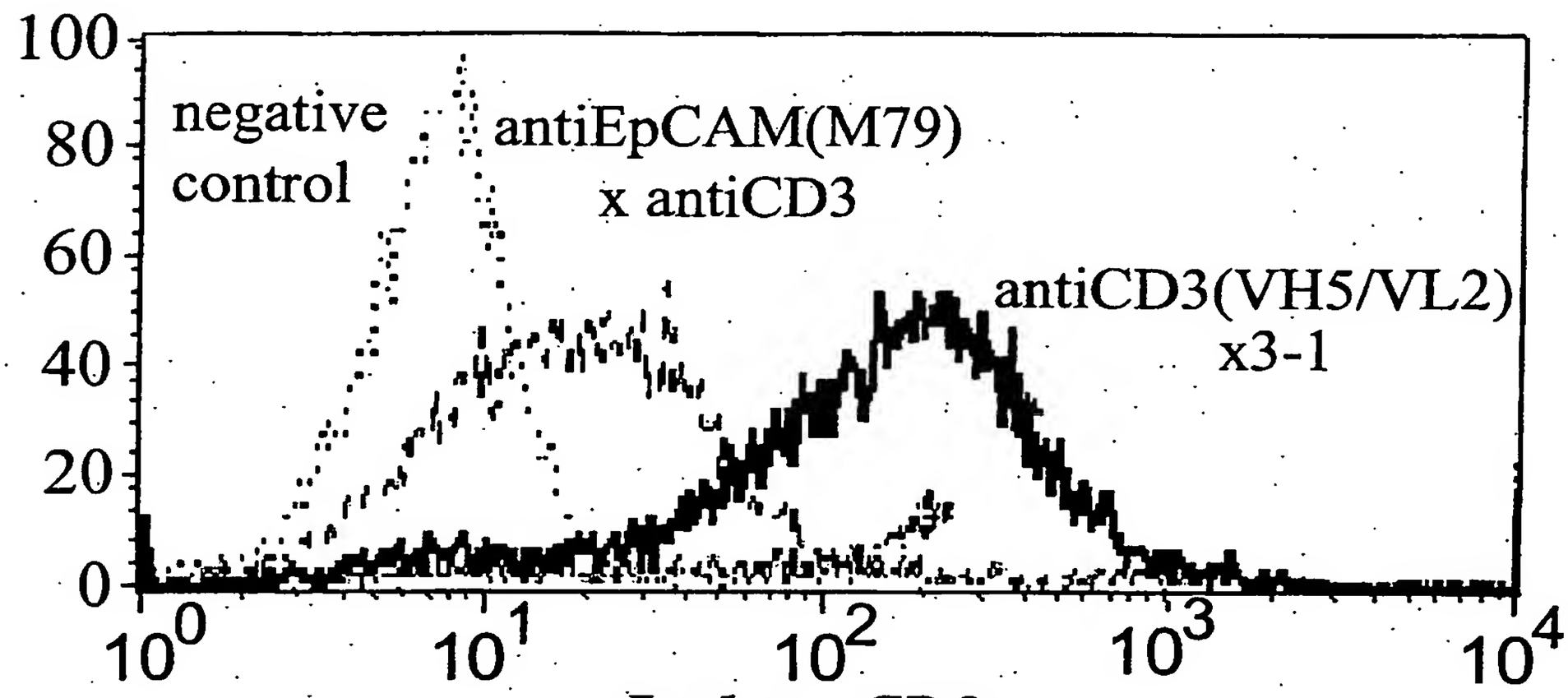
Jurkat, CD3



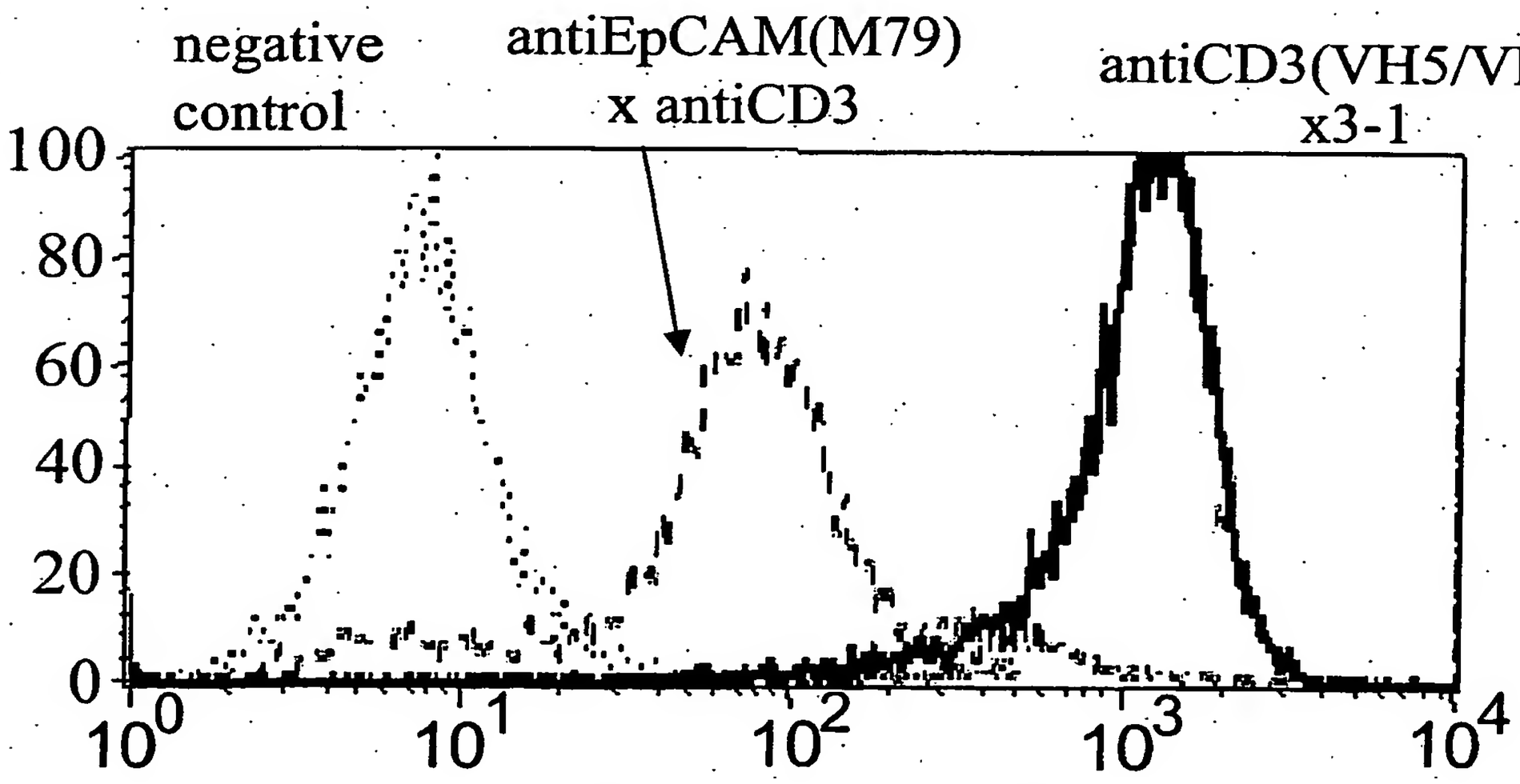
KatoIII, EpCAM

Figure 15C

antiCD3(VH5/VL2) x 3-1 (SEQ ID NO:31)



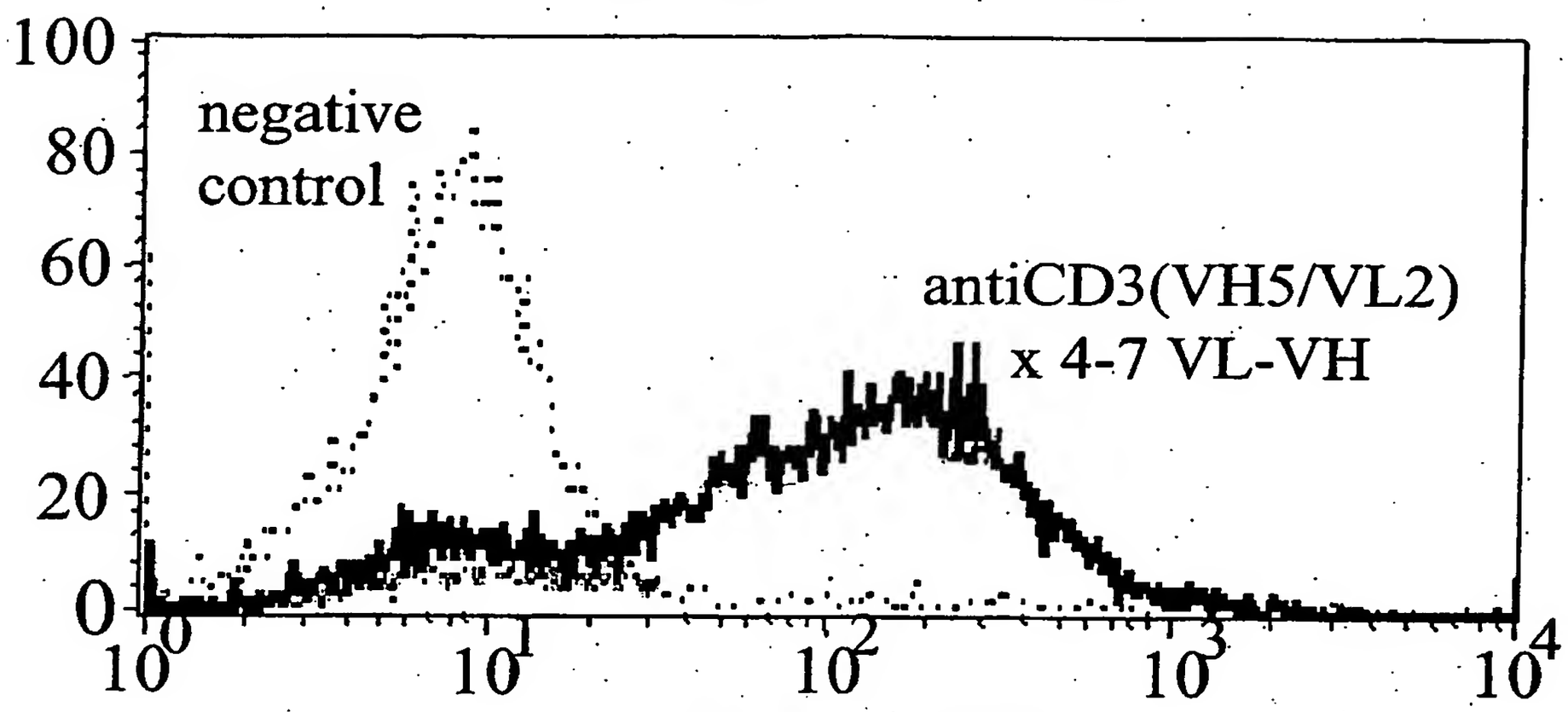
Jurkat, CD3



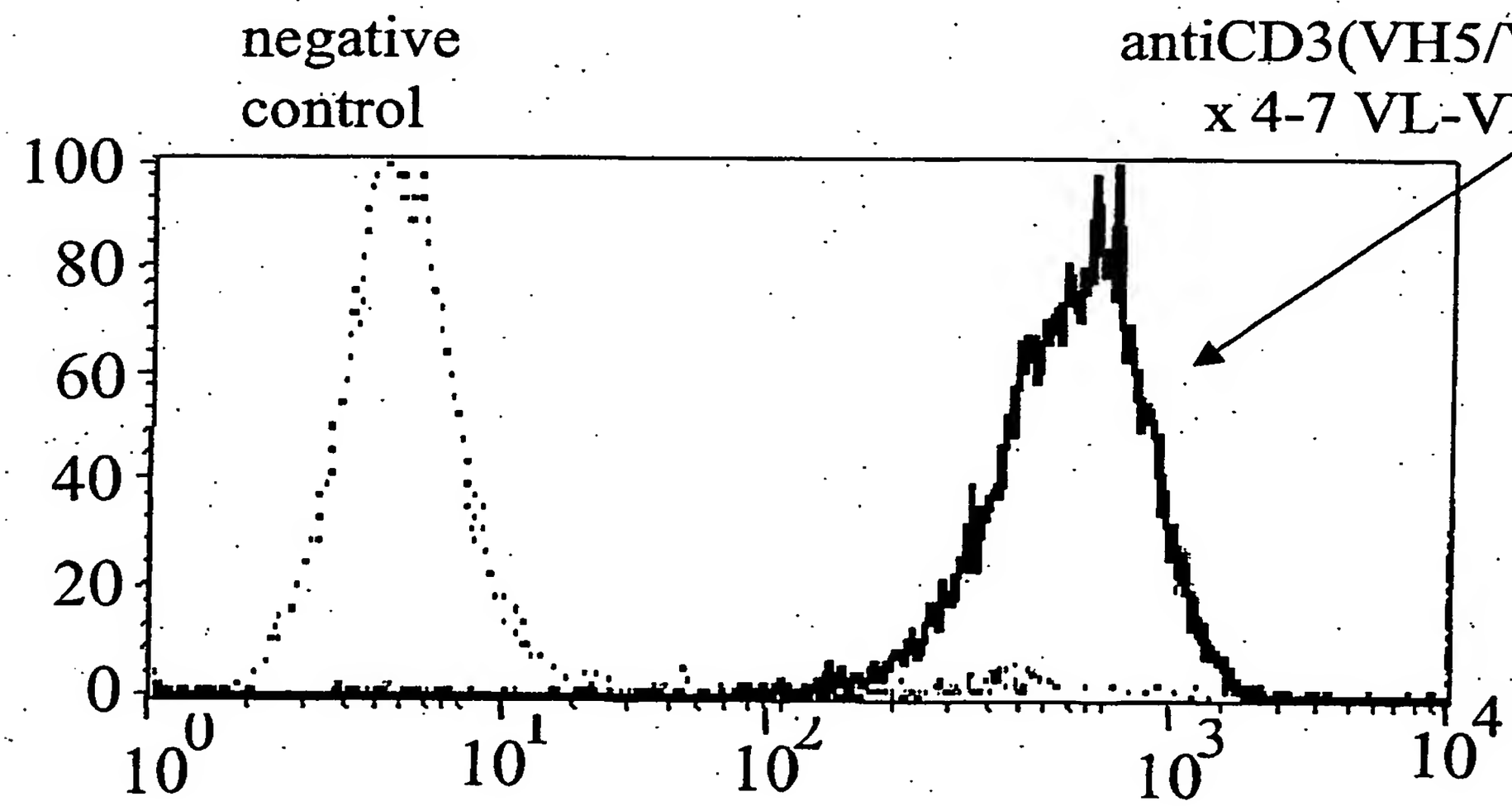
KatoIII, EpCAM

Figure 15 D

antiCD3(VH5/VL2) x 4-7 VL-VH (SEQ ID NO: 35)



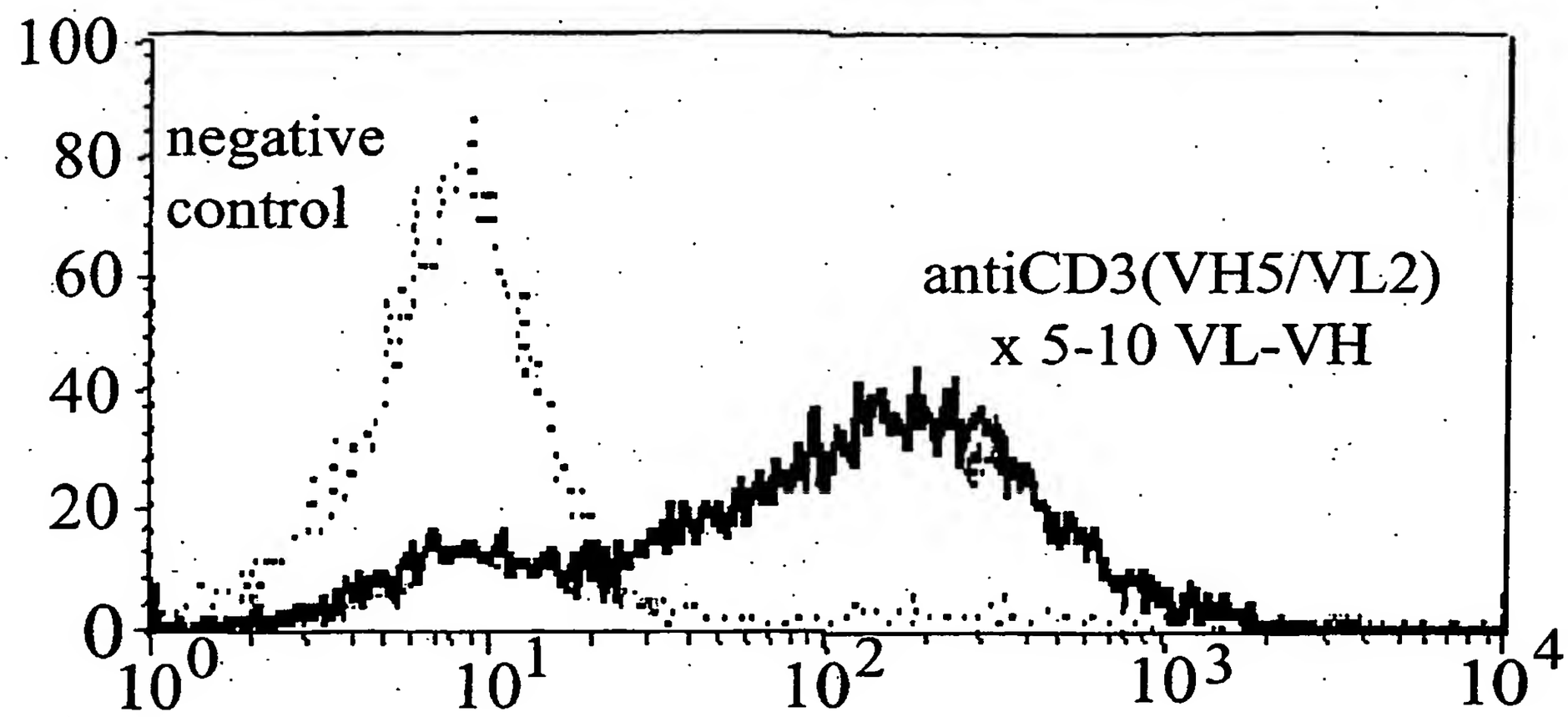
Jurkat, CD3



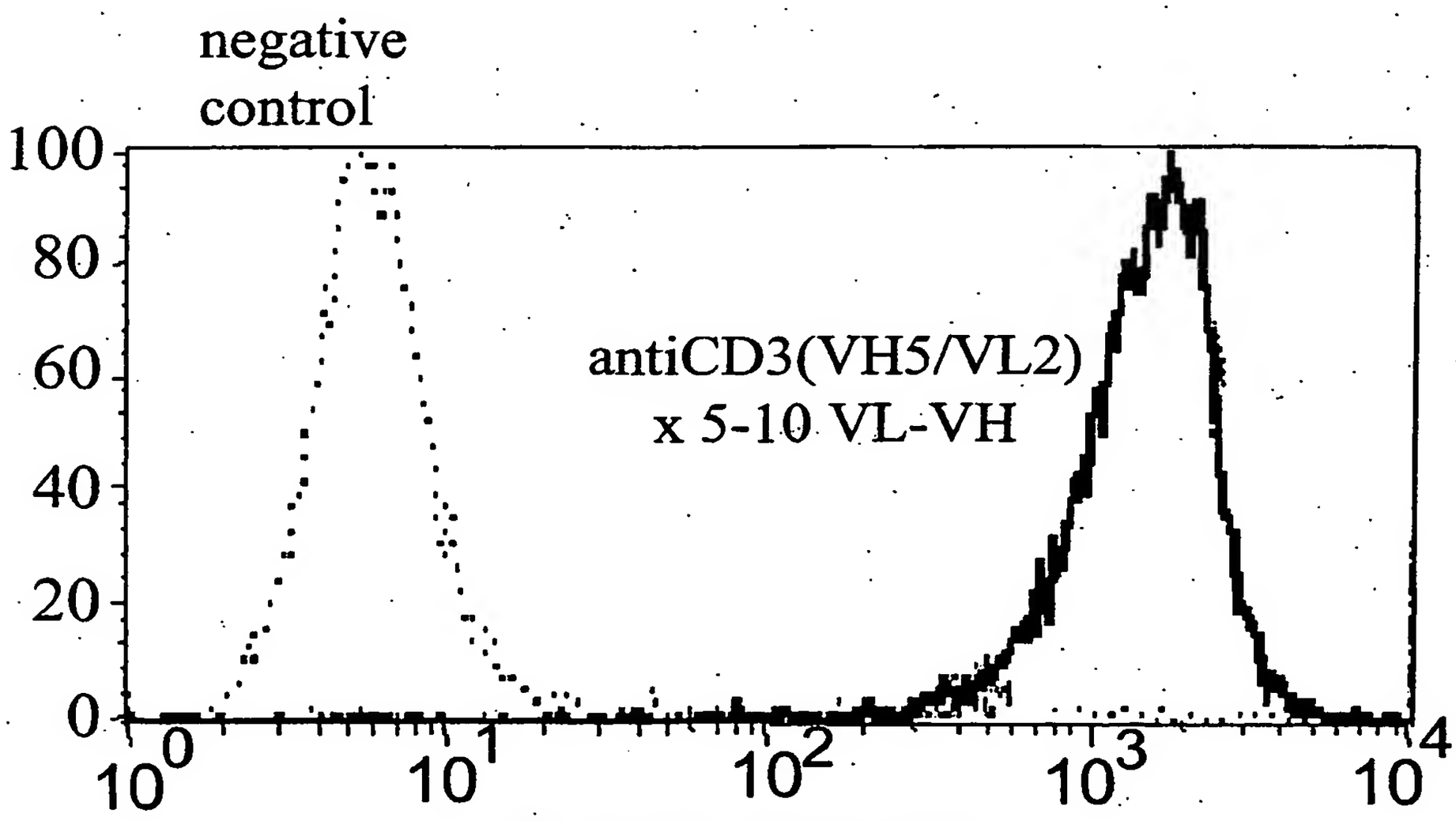
KatoIII, EpCAM

Figure 15 E

antiCD3(VH5/VL2) x 5-10 VL-VH (SEQ ID NO:39)



Jurkat, CD3



KatoIII, EpCAM

Figure 16 A

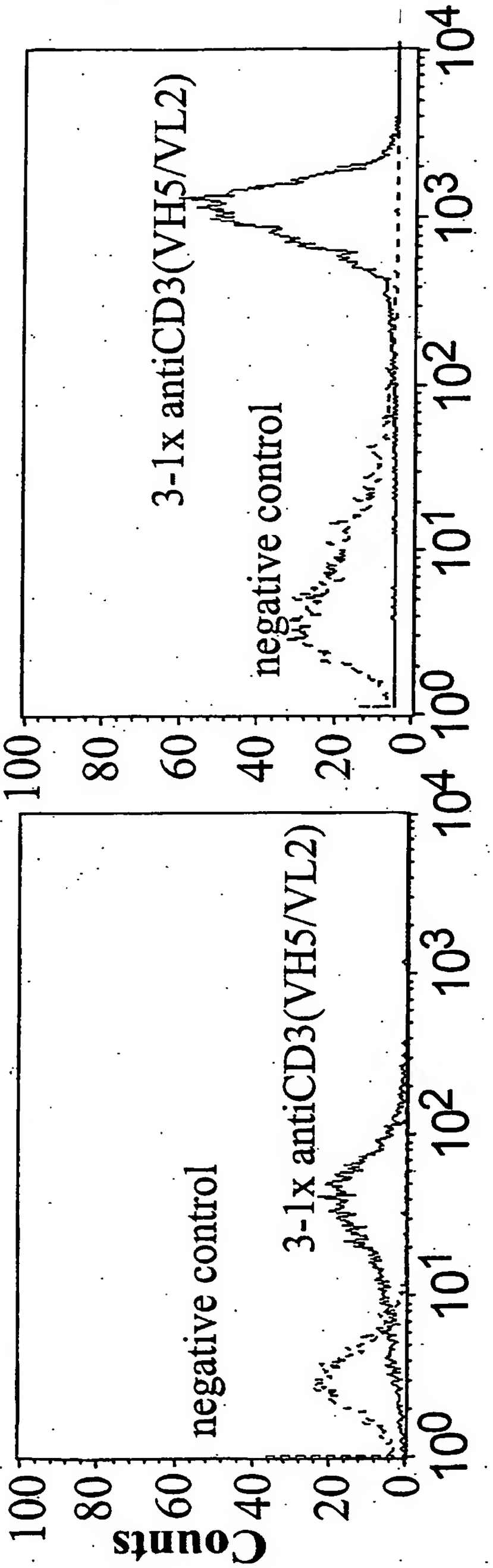

CD3 Binding (Jurkat cells)
negative control
3-1x antiCD3(VH5/VL2)
Counts
EpCAM Binding (Kato cells)
negative control
3-1x antiCD3(VH5/VL2)
3-1 x
antiCD3(VH5/VL2)
(SEQ ID NO: 49)

Figure 16 B

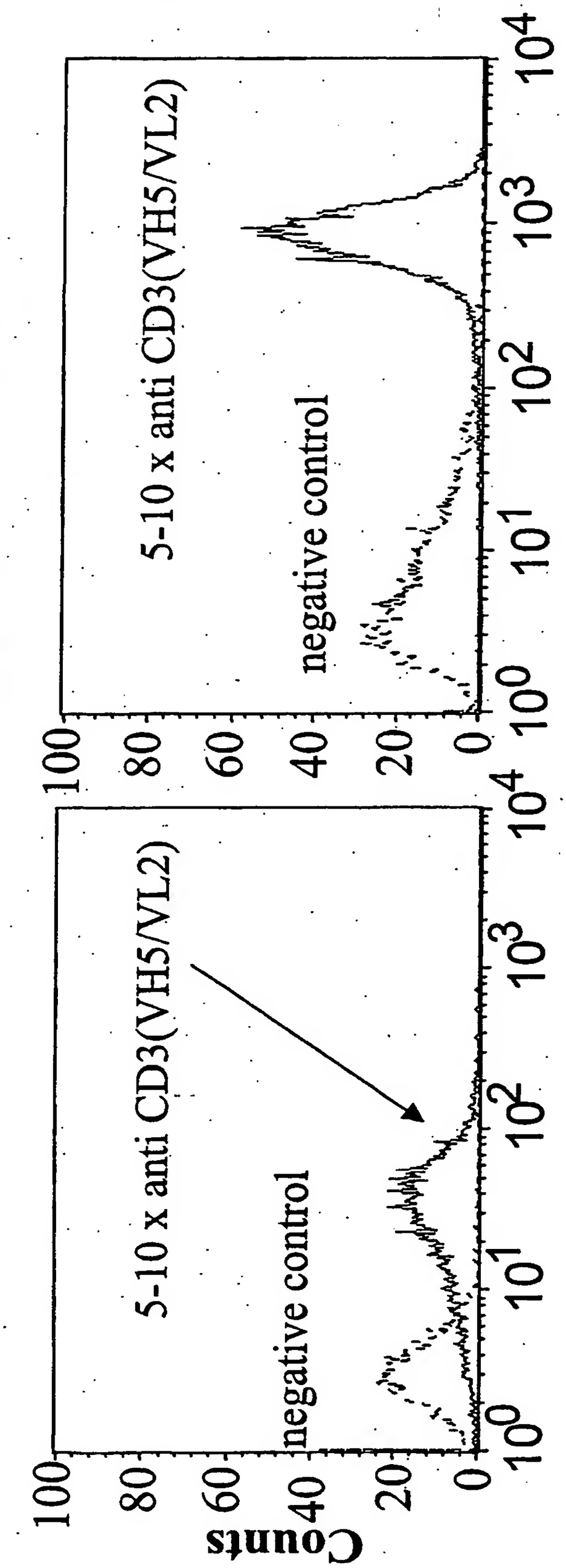

CD3 Binding (Jurkat cells)
5-10 x anti CD3(VH5/VL2)
negative control
Counts
EpCAM Binding (Kato cells)
5-10 x anti CD3(VH5/VL2)
negative control
5-10 x antiCD3(VH5/VL2)
(SEQ ID NO:63)

Figure 17

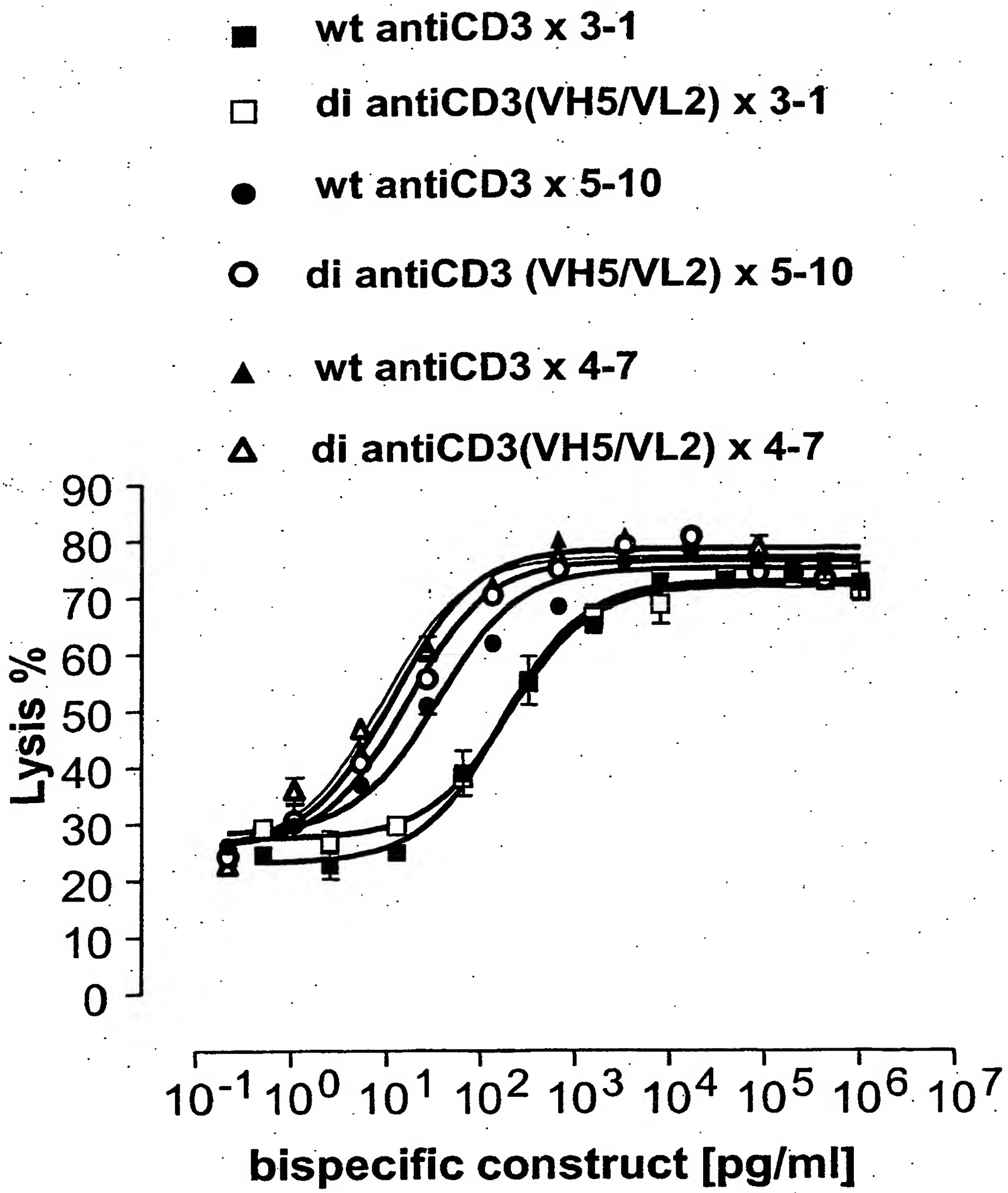
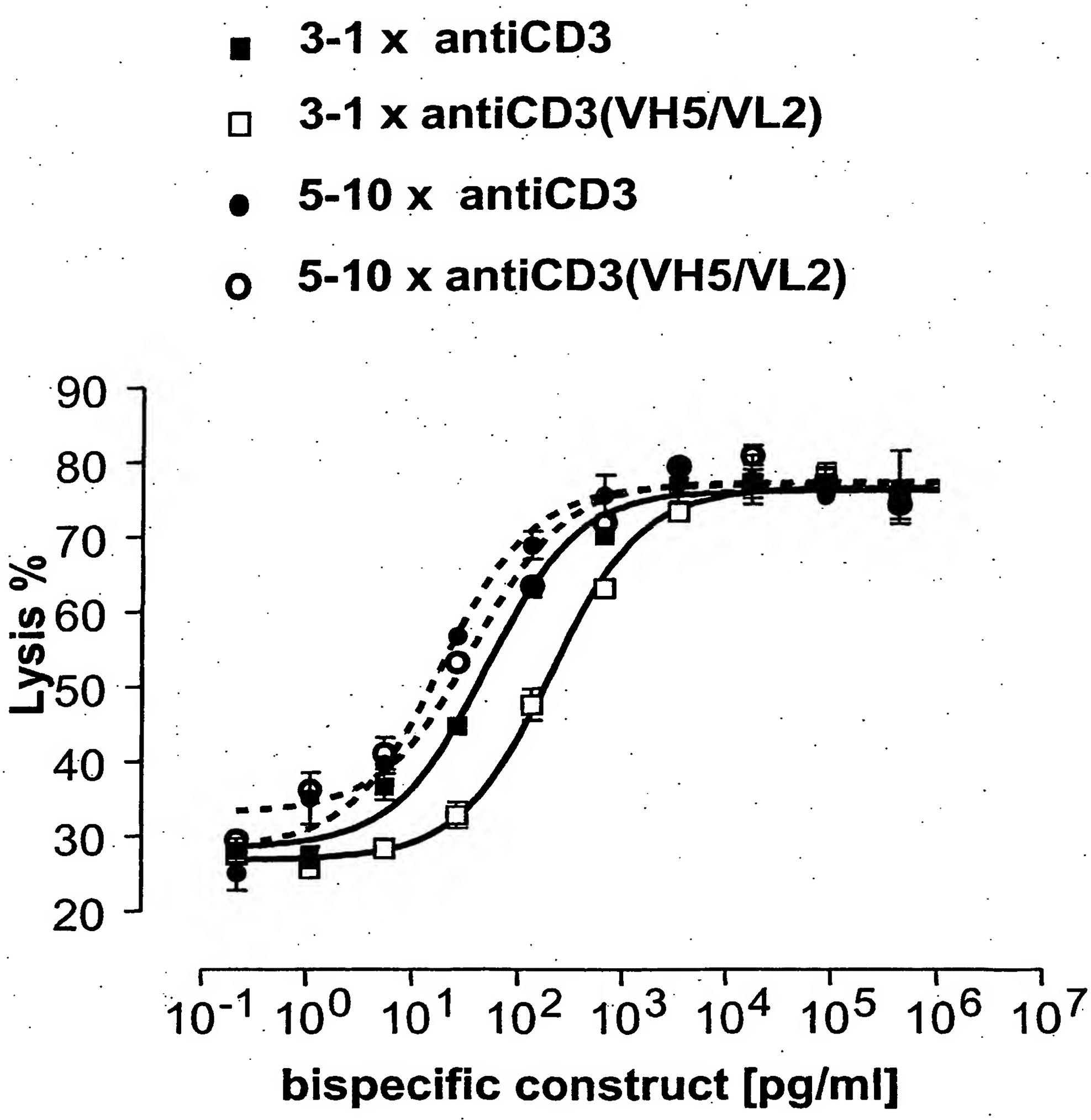

wt antiCD3 x 3-1
di antiCD3(VH5/VL2) x 3-1
wt antiCD3 x 5-10
di antiCD3 (VH5/VL2) x 5-10
wt antiCD3 x 4-7
di antiCD3(VH5/VL2) x 4-7
Lysis %
90
80
70
60
50
40
30
20
10
0
10-1 100 101 102 103 104 105 106 107
bispecific construct [pg/ml]

Figure 18


3-1 x antiCD3
3-1 x antiCD3(VH5/VL2)
5-10 x antiCD3
5-10 x antiCD3(VH5/VL2)
Lysis %
90
80
70
60
50
40
30
20
10-1 10^0 10^1 10^2 10^3 10^4 10^5 10^6 10^7
bispecific construct [pg/ml]